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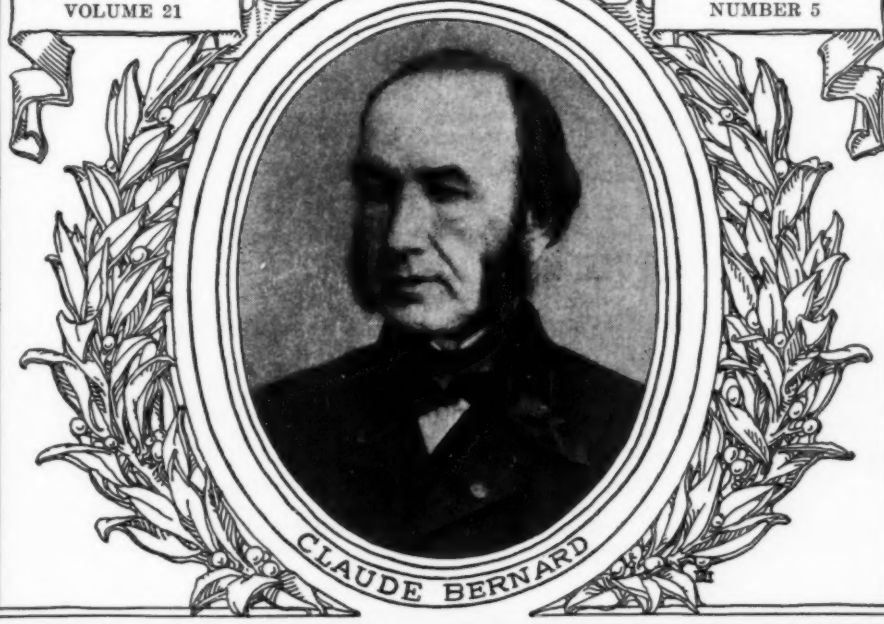

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Woodland Avenue and Thirty-sixth Street, Philadelphia, Pa.

THE EFFECT OF CERTAIN CARCINOGENS ON VITAMIN A IN THE LIVER [1]

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(Received for publication November 12, 1940)

Several observations suggest that carcinogenic compounds may alter vitamin A metabolism. The intraperitoneal injection of dibenzanthracene into rabbits reduced the vitamin A content of liver mitochondria, while p-aminoazobenzene, a non-carcinogenic ring compound, was without effect (Goerner, '38). Livers of rats fed aminoazotoluene until hepatomas appeared contained less vitamin A than normal livers, and the tumors themselves were devoid of the vitamin (Goerner and Goerner, '39 a). Rats and rabbits injected intraperitoneally with dibenzanthracene showed markedly lowered hepatic stores of vitamin A as compared to uninjected controls, although the hepatic stores of vitamins C and D, and of glutathione were unaffected by the injections (Goerner and Goerner, '39 b, c).

Unfortunately, the material used in the latter experiments was commercial dibenzanthracene, a product which until recently has been quite impure (Fieser, '38). Furthermore, the animals' source of vitamin A was a commercial chow fed ad libitum, and the food consumption of the various groups was not recorded. It is well-known that the hepatic storage of vitamin A parallels the amount of vitamin ingested, and preliminary experiments of our own have indicated that the presence of an azo dye in certain rations may reduce food intake

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by as much as 40%. The intraperitoneal injection of hydrocarbons also lowered food consumption somewhat. Much of the observed reduction in vitamin A storage may therefore have been merely the effect of a reduced food intake. Inasmuch as very little is known about factors which alter the fate of vitamin A in the body, it became desirable to determine whether dibenzanthracene could affect hepatic storage of vitamin A under suitably controlled conditions, and if so, whether this phenomenon is related to carcinogenesis.

METHODS

Rats 3 to 4 weeks of age and 35 to 50 gm. in weight were used for the experiments. They were fed a basal ration devoid of vitamin A but complete in other dietary essentials. It had the following percentage composition: casein 18, salts 4, yeast 7.9, irradiated yeast 0.1, agar 2, cottonseed oil 5, dextrinized corn starch 63. On this ration growth ceases, and ophthalmic symptoms appear in 5 weeks.

The carcinogenic agents used were dibenzanthracene, methyl cholanthrene, benzpyrene, and butter yellow (dimethylaminoazobenzene). As examples of non-carcinogenic substances 1, 2 benzanthracene, and India ink were tested. In our earlier experiments the dibenzanthracene was purified by treatment with lead tetraacetate (Fieser and Hershberg, '38) and the methyl cholanthrene by chromatographic adsorption on Al_2O_3. The resulting products were white crystals and pale yellow crystals, respectively. Commercial samples of these agents obtained later were sufficiently pure for use directly.

Colloids for injection were prepared by dissolving the agent in hot acetone and adding it dropwise to an aqueous solution of gelatin maintained at 80–90°C. The residual acetone was distilled off under suction, and the final volume so adjusted that the colloid contained 1 mg. of hydrocarbon in 1 cc. of 1% gelatin. The samples were stored in the refrigerator when not in use. Hepatic vitamin A was determined colorimetrically by the $SbCl_3$ reaction (Carr and Price, '26) and the results expressed as blue units (Moore, '30) per liver.

EXPERIMENTAL

Rate of depletion of hepatic vitamin A

In the first experiment twenty rats 35 to 50 gm. in weight were fed the basal low A ration, plus 1 drop of halibut liver oil per rat daily for 6 days. At this point the supplement of halibut liver oil was discontinued. The oil contained 1250 blue units per drop. Two rats were killed on the seventh day and analysis revealed the presence of 2955 blue units of vitamin A per liver. The surviving animals were divided into three groups. The first received three intraperitoneal injections per week of 1 mg. each of colloidal dibenzanthracene, the amount used by Goerner and Goerner ('39 b). Since this is more than need be given to induce tumors in rats, a second group was injected with 1 mg. of dibenzanthracene once a week. The third group received no injections. The animals were kept on the low A diet and were killed for analysis at 3, 8 and 10 weeks. The results are summarized in table 1.

TABLE 1

The effect of intraperitoneal injections of colloidal dibenzanthracene on the rate of depletion of hepatic vitamin A [1]

Blue units per liver

WEEKS	UNINJECTED CONTROLS	DIBENZANTHRACENE INJECTION	
		3 mg./week	1 mg./week
0	2955 (2920–2990)	2955	2955
3	3155 (3120–3190)	2300	....
8	2420 (2330–2510)	703 (584–823)	520 (473–567)
10	1267 (1180–1317)	236 (105–368)	268 (258–278)

[1] Each rat received 1250 blue units per day for 6 days previous to beginning of administration of the hydrocarbon.

Marked differences between groups were observed in the rate of depletion of hepatic vitamin A. After 8 weeks the uninjected animals still had 2400 units of vitamin A in their livers, a loss of 600 units, whereas the two groups of animals treated with dibenzanthracene had 500 and 700 units, respectively, a loss of 2300 to 2500 units. The rate of depletion of vitamin A, therefore, had been increased approximately

fourfold by the dibenzanthracene. Since the rate of depletion of the vitamin was slightly greater in animals injected once weekly than in those injected thrice weekly, 1 mg. of dibenzanthracene per week was ample to demonstrate the effect. On this level whitish strongly fluorescent residues of the hydrocarbon were invariably found in the abdominal cavity on autopsy.

Animals injected thrice weekly contained much larger residues and in addition adhesions between the liver and adjacent tissues were present. Such animals were usually very emaciated, although even with the largest injections, growth continued during the early weeks of the experiment. At 8 weeks the average weights were 193 gm. for the uninjected control animals, 139 for those receiving 1 mg. dibenzanthracene per week, and 128 for those receiving 3 mg. per week. To reduce possible complicating effects of adhesions, a standard amount of hydrocarbon, viz., 1 mg., was injected weekly in all subsequent experiments.

Absorption and storage of vitamin A

Having established that dibenzanthracene affected the mechanisms by which vitamin A leaves the liver, an attempt was made to determine whether the hydrocarbon affected the mechanisms by which vitamin A enters the liver. The following procedure was adopted: eight rats, 50 gm. in weight, were divided into two groups of four and given the low A ration. The first served as uninjected controls, whereas the second received three intraperitoneal injections of 1 mg. colloidal dibenzanthracene during 1 week. Two days after the last injection the animals of both groups were fed 4 drops of halibut liver oil each, and 2 days later still, they were killed for analysis. No abnormalities were noted in any of the animals, except for the presence of hydrocarbon in those injected. The presence of hydrocarbon markedly depressed hepatic storage. Control livers contained 2840 to 3150 blue units per liver, the average being 3040 units; livers from injected animals ranged from 1260 to 1650, with an average of 1500 units.

Since our 50-gm. animals contain but 50 to 75 blue units per liver, the above discrepancy could not represent a loss sustained during the week of injection.

Furthermore, the rate of depletion of vitamin A from livers containing 3000 units ranges from 100 to 300 units per week (table 1) rather than the 1500 units of loss per week which would have to be assumed if the effect of the dibenzanthracene were attributed only to the mechanism of depletion. It therefore appears that the injected vitamin A had also interfered with the process by which vitamin A enters the liver.

Comparison of dibenzanthracene and methyl cholanthrene

Dibenzanthracene is a relatively weak carcinogenic substance; methyl cholanthrene, one of the most potent carcinogenic agents known. If the effect of dibenzanthracene on vitamin A metabolism were related to tumor formation, then the more potent carcinogen, methyl cholanthrene, might be expected to show an even greater effect on hepatic vitamin A. The two hydrocarbons were therefore compared for their overall effect on vitamin A metabolism. Three groups of seven rats each, 40 gm. in weight, were placed on the low A diet and fed halibut oil by dropper. The oil was diluted with cottonseed oil so that 1 drop contained 200 blue units of vitamin A, and each rat was fed 3 drops per week throughout the experiment. The first group received no injections; the second received 1 mg. colloidal dibenzanthracene intraperitoneally once a week; the third received 1 mg. colloidal methyl cholanthrene once a week. The animals were killed for analysis at intervals. All animals grew well and no abnormalities appeared at autopsy. The results are summarized in table 2. Although both hydrocarbons reduced vitamin A stores, methyl cholanthrene caused less rather than more depletion than benzanthracene. Considering the animals killed at different times collectively, the uninjected controls averaged 1887 blue units per liver, the methyl cholanthrene animals averaged 892 and the dibenz-

anthracene animals 489 blue units per liver. Hence, dibenzanthracene was about twice as effective in reducing hepatic vitamin A as the more potent carcinogen, methyl cholanthrene.

TABLE 2

The action of methyl cholanthrene and of dibenzanthracene on hepatic vitamin A [1]

Blue units per liver

WEEKS OF EXPERIMENT	UNINJECTED CONTROLS	DIBENZANTHRACENE	METHYL CHOLANTHRENE
4½	1551	546	922
5	1452	462	953
5½	1980	410	850
			670 [2]
6	2310		760 [2]
	1782	861	1250 [2]
6½	2250	300	840
		345	
		498	
Average	1887	489	892

[1] Six hundred blue units of vitamin A were given to all animals weekly. The animals averaged 75 blue units of vitamin A per liver at the beginning of the experiment.

[2] Animals died.

Effect of other compounds

The effect of other compounds on hepatic vitamin A was next investigated. Young rats 50 to 60 gm. in weight were placed on the low A ration, fed 6 drops of halibut liver oil during the first 6 days, and then continued on the low A ration. Except for group 6 they were injected intraperitoneally once a week with 1 cc. of colloidal carcinogen, and were killed for analysis at 8 weeks. Group 6 received 1 cc. of colloidal dibenzanthracene every other week, and was likewise killed after 8 weeks. The compounds, numbers of injections, numbers of animals, and the vitamin A content of the livers after 8 weeks are listed in table 3.

Colloidal butter yellow, dimethylaminoazobenzene, was practically devoid of any effect on hepatic vitamin A. This compound is of particular interest because its carcinogenic

action does not occur at the site of administration, like the hydrocarbons, but whether fed or injected it produces tumors in the liver instead (Kinosita, '40). Its failure to affect liver stores of vitamin A would appear to indicate that the production of hepatomata, and the storage and unloading of hepatic vitamin A are distinct and unrelated processes, even though they both occur in the same organ.

TABLE 3

The effect of various carcinogenic agents on the hepatic vitamin A content of rats maintained for 8 weeks on a low A diet

COMPOUND	NUMBER OF INJECTIONS	NUMBER OF ANIMALS	RANGE: HEPATIC VITAMIN A	AVERAGE BLUE UNITS/LIVER
			blue units	
Control	0	4	3100–4120	3700
Butter yellow	8	4	2200–4300	3425
Benzpyrene	8	4	2460–3530	2990
Benzanthracene[1]	8	4	1800–3600	2760
Methyl cholanthrene	8	6	1600–2800	2100
Dibenzanthracene	4	4	1740–3000	2340
Dibenzanthracene	8	3	1440–1560	1490

[1] This compound is non-carcinogenic, although related chemically to many carcinogens.

Benzpyrene and 1, 2 benzanthracene accelerated the depletion of hepatic vitamin A to about the same extent. Livers of animals receiving these compounds contained 2990 and 2760 blue units of vitamin A respectively, as compared to 3700 for the uninjected controls (table 3). This similarity is particularly striking in view of the very great differences in the tumor-producing power of the two hydrocarbons, benzpyrene being a potent carcinogen whereas 1, 2 benzanthracene is practically devoid of carcinogenic activity (Fieser, '38). Evidently, therefore, the effect of a hydrocarbon on vitamin A does not necessarily parallel its carcinogenic potency.

Animals receiving eight injections of methyl cholanthrene contained 2100 blue units per liver; those receiving four injections of dibenzanthracene contained 2340 (table 3). In other words, about twice as much methyl cholanthrene as

dibenzanthracene was needed to produce a comparable effect on vitamin A storage, again illustrating the discrepancy between carcinogenic potency and effect on hepatic vitamin A. Animals receiving eight injections of dibenzanthracene had the lowest stores of all, 1490 blue units. Incidentally in another series it was shown that colloidal carbon black (India ink diluted 1:8 with water) was without effect on hepatic vitamin A.

The effects of the compounds on hepatic vitamin A appear to be metabolic. They are not artifacts of the chemical determination, since the addition of any of the hydrocarbons, or of butter yellow, to the $SbCl_3$ reaction mixture failed to alter the intensity of the blue color produced. In seventeen experiments various hydrocarbons were added to minced liver, incubated for periods of time up to 4 hours, and the tissue digested and analyzed as before. In no case did samples treated with hydrocarbon differ in vitamin A content from comparable aliquots free of hydrocarbon.

Effect of carcinogenic amounts of hydrocarbons

In all previous experiments hydrocarbons were injected intraperitoneally because this was the method used by Goerner ('38), and Goerner and Goerner ('39 a, b, c) and because the technique readily produced changes in hepatic vitamin A. However, this is not the usual method employed in studying tumor production with these agents. No tumors appeared during the studies on vitamin A storage because the experiments lasted for only 10 weeks or less, whereas at least 4 months, and sometimes as much as a year, are needed to produce tumors in rats with these hydrocarbons (Fieser, '38; Rusch, Baumann and Maison, '40). A "carcinogenic method" of administering the hydrocarbon was therefore employed, and the effect on vitamin A storage determined.

Methyl cholanthrene and dibenzanthracene were dissolved in lard (8 mg./cc.) and ½ cc. was injected subcutaneously, twice the first month, and once monthly thereafter. Three groups of ten rats each were used. One group received lard

only; the others methyl cholanthrene and dibenzanthracene respectively. The animals averaged 65 gm. in weight at the beginning of the experiment when they were placed on the low A diet and given 2 drops of halibut liver oil daily for 3 weeks. Thus enough hepatic vitamin A was accumulated to supply the needs of the animals until tumors appeared. Animals were killed for analysis at intervals. After 18 weeks, all of the survivors treated with methyl cholanthrene had palpable tumors,[2] the largest being 4×5 cm. in diameter. No tumors appeared in the other groups.

The livers of animals killed during the nineteenth week contained the following amounts of vitamin A:

Controls	3250 blue units, range 1710–5570
Methyl cholanthrene	2500 blue units, range 2165–2810
Dibenzanthracene	1320 blue units, range 1150–1530

In view of both the large amounts of vitamin A originally administered and the long depletion period, wide variations within groups might be expected. Nevertheless, several conclusions are warranted by the data: (1) dibenzanthracene lowers hepatic vitamin A whether the hydrocarbon is given subcutaneously in oil or intraperitoneally as a colloid; (2) given in oil, dibenzanthracene exerts more effect on vitamin A than methyl cholanthrene; (3) even the actual presence of tumors due to methyl cholanthrene failed to produce any very considerable reduction in the liver stores of the vitamin.

Effect of spontaneous tumors on vitamin A stores

Our strain of rats is subject to the development of spontaneous mammary adenofibromas.[2] These tumors are slow-growing, encapsulated, and frequently attain an enormous size without either invading the body wall or becoming necrotic. The tumor contains much connective tissue. Analyses of the livers of adult rats bearing tumors which weighed from 7

[2] We are indebted to Dr. H. P. Rusch of the McArdle Memorial Laboratory, University of Wisconsin, for the examination of these tumors.

to 100 gm., showed a range of 7000 to 17,000 blue units of vitamin A per liver, average 12,800. The variations could not be related to tumor size. Analyses of fourteen livers from stock rats of similar age showed ranges of 4500 to 13,100 blue units per liver, average of 8200 blue units. The latter figure is somewhat greater than the result of a survey made 7 years ago, when the average in the colony was found to be 6000 blue units (Baumann, Riising and Steenbock, '34).

The presence of even a very large tumor, therefore, does not result in a reduction of hepatic vitamin A, as experiments with dibenzanthracene might lead one to suppose. On the contrary, the stores may actually be increased if the tumor-bearing rats consume more food and hence more vitamin A. However, a diet low in vitamin A has been reported to cause a benign type of growth, odontoma, in rat incisors (Orten, Burn and Smith, '37), although low A diets did not affect tumor production due to benzpyrene (Howe, Elliot and Shear, '40). Sure, Buchanan and Thatcher ('36) have shown that enormous doses of carotene do not affect tumor growth.

Deficiency symptoms

The effectiveness of dibenzanthracene in reducing liver stores of vitamin A suggested that the hydrocarbon might hasten the appearance of deficiency symptoms. This proved to be the case with two groups of twelve rats each, which were placed on the low A diet when 40 to 45 gm. in weight, the rats of one group being injected with 1 mg. dibenzanthracene weekly, and the others serving as controls. Ophthalmia appeared in the injected animals on an average of 3 days sooner than in those not injected, and the growing period was shortened by 9 days (30 as compared to 39). The rate of growth was markedly retarded by the hydrocarbon, a phenomenon which, however, is also observed when adequate amounts of vitamin A are present. Attempts at comparing the vaginal smear responses (Baumann and Steenbock, '32) were unsuccessful, because the vaginal orifice of the injected animals failed to open.

DISCUSSION

Whatever the explanation or the mechanism involved, it is evident that numerous hydrocarbons tend to reduce the storage of vitamin A in the liver, and furthermore, that the dibenzanthracene molecule possesses this property to a very high degree. The phenomenon is apparently unrelated to tumor formation, in spite of the fact that certain compounds (DBA, MC, BP) are active both as carcinogens and as "anti-storage agents." However, 1, 2 benzanthracene is an example of a compound which reduces vitamin A storage without being carcinogenic, whereas butter yellow is a carcinogen which does not reduce hepatic vitamin A. The situation might be compared to the relationship between certain estrogens and carcinogens. Here too, some molecules, such as benzpyrene and estrone, possess both properties, but their activities do not run parallel, and examples can be cited of compounds which show only the one or the other activity (Cook et al., '34; Lacassagne, '36).

In general, most recorded attempts at lowering hepatic vitamin A by physiological techniques have been unsuccessful unless the absorption of the vitamin was disturbed. Thus, vitamin A storage is normal in many clinical disorders (Moore, '37; Ellison and Moore, '37; Wolff, '32) as well as in the experimental loss of liver fat due to starvation or vitamin B_1 deficiency (Dann and Moore, '31), or the choline-induced loss of liver fat (Lease and Steenbock, '39). Liver damage due to phosphorus, $CHCl_3$ or CCl_4 apparently does not alter vitamin A storage (Lasch, '35; Wendt and Koenig, '37; Greaves and Schmidt, '35, '36). Furthermore, the alleged antagonism between vitamin A and thyroxin has not been universally accepted (Logoras and Drummond, '38; Baumann and Moore, '39). On the other hand, reduced storage of vitamin A has been observed when the reticulo-endothelial system was blocked with thorotrast (Lasch and Roller, '36), phenylhydrazine, or bismuth (Wendt and Koenig, '37). Alcohol, likewise, is said to reduce the storage of vitamin A

(Ikegaki, '38). It is to these latter substances that the action of the hydrocarbons should be compared, even though the mechanism involved is as yet unknown.

SUMMARY

1. Colloidal dibenzanthracene, injected intraperitoneally, increased the rate of depletion of hepatic vitamin A fourfold, and also appeared to interfere with the entry of vitamin A to the liver. Dibenzanthracene injected subcutaneously in oil also increased vitamin A depletion.

2. Methyl cholanthrene and benzpyrene, two potent carcinogens, and 1, 2 benzanthracene, a non-carcinogenic hydrocarbon, likewise reduced hepatic vitamin A, but all three were quantitatively less effective than dibenzanthracene. There was therefore no correlation between the carcinogenicity of a compound and its effect on vitamin A. Butter yellow, which is carcinogenic, and carbon black, which is non-carcinogenic, were without effect on the vitamin.

3. The livers of rats with spontaneous tumors contained more vitamin A than the livers of non-tumorous controls. Livers of rats with tumors due to methyl cholanthrene contained more vitamin A than non-tumorous rats treated with dibenzanthracene. Decreased vitamin A, therefore, is not a necessary prerequisite to tumor formation.

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THE SPARING ACTION OF THIAMINE ON BODY TISSUE CATABOLISM [1]

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During 1931–1932 this laboratory published a series of papers in which data were presented, showing that vitamin B_1 exerts a per se influence on growth, as evidenced by the paired-feeding method of experimentation. Such conclusion is in accord with that of Mitchell ('33) and with those of Graham and Griffith ('33), but it is at variance with those of Hogan and Pilcher ('33) and McClure, Voris and Forbes ('34). Since in the years 1931 to 1934 vitamin B concentrates were employed as a source of thiamine, it is possible that the former produced an influence on growth by furnishing several components of the vitamin B complex. The earlier work, therefore, needed reinvestigation; consequently new experiments were carried out, using the technique previously described (Sure and associates, '31 and '32) with the following modifications. The feeder employed was that introduced by Swift, Kahlenberg, Voris and Forbes ('34) which we had constructed with heavy wire prongs to discourage animals from sitting on the lid of the feeder or from attempting to crawl inside the feeding cup. As the animals grew we found it necessary to increase the size of the feeder. The food was weighed every other day on an agate bearing beam balance to an accuracy of 10 mg., and transferred to the food containers,

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according to the technique of Hogan and Pilcher ('33). No difficulties were encountered with this type of feeder in obtaining accurate quantitative records of food consumption. Any small particles of food spilled on the platform of the feeder were carefully transferred to the cup with the aid of a fine brush.

EXPERIMENTAL

The results of this investigation are presented in tables 1 and 2. The experiments were carried out in four series.

Series I. This study was conducted on ten pairs of rats subsisting on a purified diet with the following percentage composition: casein (vitamin-free)[2] 18; salt mixture no. 351 (Hubbell, Mendel and Wakeman, '37) 4; agar-agar 2; nicotinic acid 0.05; hydrogenated cottonseed oil [3] 10; dextrin 65.95. This was supplemented daily with 20 μg. riboflavin, 20 μg. pyridoxin, 6 mg. choline, and 0.2 cc. to 0.5 cc. of a solution containing the "W" factor, equivalent to 0.2 and 0.5 gm. liver extracts respectively. The "W" factor extracts provided 30 to 75 μg. pantothenic acid daily as well as other unidentified filtrate factors essential for growth. The control animals received in addition 20 μg. thiamine daily. Each animal received daily except Sundays 6 drops of cod liver oil as a source of vitamins A and D. On such a diet fed to control animals ad libitum excellent growth was secured; but when the control rats were restricted to the diminishing food intakes of the thiamine deficient animals, during an average experimental period of 50 days, they lost an average of 4.5 gm. per rat. The thiamine deficient animals, however, lost an average of 19.1 gm. per rat, or over 400% more than the litter mate controls. In other words, the thiamine administered to the control animals produced a pronounced sparing effect on body tissue catabolism (table 1).

[2] Supplied by the Borden Company, New York, under the trade name of "Labco."

[3] Crisco.

TABLE 1

The role of thiamine in body tissue catabolism (ration 11)

PAIR NUMBER [1]	PERIOD OF EXPERIMENTATION	INITIAL WEIGHT	FINAL WEIGHT	CHANGE IN WEIGHT
	days	*gm.*	*gm.*	*gm.*
1 — P	58	95	87	—8
RC		98	89	—9
2 — P	57	86	83	—3
RC		90	91	+1
3 — P	36	84	62	—22
RC		87	67	—20
4 — P	48	96	70	—26
RC		87	83	—4
5 — P	62	95	76	—19
RC		95	78	—17
6 — P	52	106	71	—35
RC		104	96	—8
7 — P	54	86	84	—2
RC		90	108	+18
8 — P	54	84	76	—8
RC		92	97	+5
9 — P	37	102	62	—40
RC		98	86	—8
10 — P	42	88	60	—28
RC		84	81	—3
Average change in weight per group during an average experimental period of 50 days.			P	—19.1
			RC	—4.5

[1] P = pathological; RC = restricted control. Restricted control animals received 20 μg. B_1 daily.

Series II. These experiments, as well as those of series III and IV, were conducted on ration 9 which has the following percentage composition: casein,[4] 16; salts (Hubbell, Mendel and Wakeman, '37), 4; autoclaved baker's yeast, 7; autoclaved beef, 8; hydrogenated cottonseed oil, 9; and dextrin, 56. Realizing, however, that riboflavin and pyridoxin may also suffer some destruction during autoclaving, in a number of experiments, either riboflavin or pyridoxin or both were given in supplementary doses of 10 μg. to the pathological and control animals. The control rats, in addition to receiving

[4] Thoroughly extracted with acidulated water and dilute ethyl alcohol.

every other day the same amount of food as the litter mates consumed the previous 48 hours, were given separately from the ration 20 μg. thiamine daily in a diluted solution in caster cups. Each animal received daily except Sundays 6 drops of cod liver oil as a source of vitamins A and D. Fourteen pairs of animals were used and the average experimental period was 42 days. Five pathological animals in this series developed marked convulsions during the terminal stages of the thiamine avitaminosis. During this stage there was an onset of marked anorexia and the body tissue catabolism in the polyneuritic animals was much greater than in the litter mate controls. To conserve space, detailed data are deleted. Further evidence on the role of thiamine in its sparing action on body tissue catabolism in this deficiency disease is presented in table 2 which shows the results of series III experiments.

It has been our experience during the past 12 years that when yeast and beef or both are autoclaved at the natural pH of these products at 15 pounds pressure, frequently appreciable amounts of thiamine are left, as evidenced by the minute amounts of supplementary crystalline thiamine that are needed daily to produce continuous growth. For this reason, we now autoclave the baker's yeast as well as the beef at 20 instead of 15 pounds pressure.

Series III. The next procedure adopted was aimed at determining whether increased amounts of thiamine given as supplements to the same food intake will produce a greater economy of food utilization. For this investigation ration 9 was used because it had been found to require only minute amounts of supplementary thiamine to permit slow but continuous growth. The thiamine was administered in daily doses of 0.1 μg. to 1.0 μg. following maintenance until it was apparent that growth could not be checked even with the small daily doses. A vitamin depletion period was then begun. The thiamine was not given from the fiftieth to the fifty-eighth day of experimentation, which allowed a depletion period of about one-half of the entire experimental period. The pathological animals received a total ranging from 0.5 to 16.4 μg.

thiamine during the entire experimental period. Since the control animals received 20 μg. thiamine daily, they received during 50 to 58 days a total of 1000 to 1160 μg. thiamine during the same interval of study. Yet, when a comparison is made between initial and maximum weights of the control and pathological animals, no differences in growth are apparent. In other words, 70 to 2000 times the amounts of thiamine as supplements to the same daily food intake produced no greater economy in food utilization. When, however, during the second half of the experimental period the thiamine was withheld from all rats, all animals lost weight, but the losses were much more pronounced in those rats which received the small doses of thiamine than in the control litter mates which received much larger allowances of this vitamin. There was an average loss of over 45% more body weight in the pathological than in the control rats during the thiamine depletion period. During the latter period the food consumption decreased 50 to 100% which, of course, accounts for the body tissue catabolism, but it is quite clear from the results of the experiments of this series that the greater intake of thiamine exerted a pronounced influence in sparing tissue catabolism during periods of inanition produced by this vitamin deficiency.

The failure of McClure, Voris and Forbes ('34) to find a function for thiamine in the economy of food utilization can now be explained by the fact that, with their experimental procedure, the pathological animals received an occasional unit of thiamine, which allowed slow growth.

Series IV. The next procedure was designed to determine the daily dose of thiamine required to keep the pathological animals as near maintenance as possible on ration 9 (with baker's yeast of this ration autoclaved at 20 pounds instead of 15 pounds pressure) supplemented with daily allowances of 10 μg. riboflavin and 10 μg. pyridoxin. The control rats received in addition 20 μg. thiamine daily. It was determined that approximately 0.05 μg. thiamine daily was required. When, however, there was an indication of slow growth, the thiamine

TABLE 2

The role of thiamine in body tissue catabolism (ration 9 with minute amounts of thiamine to the pathological animals)

RAT NUMBER [1]	PERIOD OF EXPERIMENTATION	INITIAL WEIGHT	MAXIMUM WEIGHT	LOSS IN WEIGHT FOLLOWING THIAMINE DEPLETION	TOTAL THIAMINE GIVEN PATHOLOGICAL ANIMALS DURING ENTIRE EXPERIMENTAL PERIOD
	days	*gm.*	*gm.*	*gm.*	*micrograms*
25 — P	103	70	108	33	6.5
RC		68	104	12	
26 — P	114	54	158	46	1.8
RC		50	166	44	
27 — P	103	58	121	36	0.5
RC		55	130	20	
28 — P	103	56	122	28	0.6
RC		64	130	24	
29 — P	100	60	103	27	0.5
RC		56	110	14	
30 — P	103	58	128	33	0.6
RC		54	133	15	
31 — P	109	70	142	28	12.2
RC		67	127	23	
32 — P	104	75	170	47	16.4
RC		73	172	33	
33 — P	105	66	151	33	12.4
RC		68	160	26	
34 — P	94	59	132	38	8.9
RC		54	125	21	
35 — P	94	54	161	53	8.6
RC		50	172	44	

[1] P = pathological; RC = restricted control. Restricted control animals received 20 μg. B_1 daily.

was withheld, or if a definite decline in growth was threatened, the daily dose was temporarily increased to 0.1 μg. In seven pairs thus studied, so long as the deficient animals were kept at maintenance with minute amounts of thiamine, the control rat, which received 100 times the amount of this vitamin (on the same food intake) showed no greater growth; that is, at an approximately maintenance level, greater amounts of thiamine produced no influence on economy of food utilization.

Just how thiamine functions in its influence in sparing body tissue catabolism is not clear from the data submitted. It is now accepted that thiamine acts in the animal body as a catalyst, necessary for the degradation of pyruvate after it has been converted into its diphosphate ester, cocarboxylase (Banga, Ochoa and Peters, '39). Possibly its beneficial influence is associated with its role in intermediate carbohydrate metabolism. This is a problem that deserves further investigation.

SUMMARY

When inanition with its loss in body weight is produced by thiamine deficiency, greater intakes of this vitamin produce a sparing influence on body tissue catabolism. The mechanism of such action is not clear from the data submitted; it is suggested that this may be associated with the role thiamine plays in intermediate carbohydrate metabolism.

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RIBOFLAVIN AS A FACTOR IN ECONOMY OF FOOD UTILIZATION [1]

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Since we could find no evidence in the literature on the role of uncomplicated riboflavin deficiency in the economy of food utilization, a study was made of this problem, the results of which are submitted in this communication. The technique of animal feeding was the same as that described in the preceding paper (Sure and Dichek, '41). The study was conducted in two series of experiments on two types of diets. The first was ration 10, which is a modification of the riboflavin deficient diet of Day and Darby ('38), and has the following percentage composition: Casein,[2] 18; salts 351, 4;[3] hydrogenated cottonseed oil,[4] 8; cod liver oil, 2; and dextrin, 68. The dextrin carried an 80% alcoholic extract of 25 gm. rice polishings in 100 gm. of ration, as a source of all the components of the vitamin B complex with the exception of riboflavin. The control animals were restricted by 2-day intervals to the same ration and the same amount of food consumed by the riboflavin deficient litter mates, and in addition received 20 μg. crystalline riboflavin daily.

It was found early in this investigation that, after 40 to 50 days on ration 10, a pyridoxin anorexia develops. At a point

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[2] Thoroughly washed with acidulated water and dilute ethyl alcohol.

[3] Sure and Dichek ('41), page 446.

[4] Crisco.

when the riboflavin deficient animals are in a marked state of inanition the supplementary administration of 10 μg. pyridoxin daily to such pathological rats is accompanied by an increase in food consumption and growth. As a matter of fact, it was not possible to demonstrate the beneficial effect of riboflavin in economy of food utilization unless ration 10 was supplemented with pyridoxin, which was done in all the experiments of series I. It was thought that following the marked pyridoxin anorexia the decreased amount of food intake might not furnish a sufficiency of thiamine supplied by the rice polish extract in the ration; hence, this ration was supplemented for both the pathological and control animals with 10 μg. thiamine as well as 10 μg. pyridoxin daily.

The second series of experiments was carried out with ration 11, the composition of which has already been given (Sure and Dichek, '41). Ration 12 differed from 11 only in that it contained 0.1 instead of 0.05% nicotinic acid. Rations 11 and 12 were supplemented daily with 20 μg. thiamine, 20 μg. pyridoxin, 6 mg. choline, and 0.2 cc. of a concentrate of "W" factor, equivalent to 0.2 gm. of liver extracts as a source of the filtrate factors. The control animals, which were restricted to the same ration and same amount of food as the pathological litter mates, received in addition 20 μg. crystalline riboflavin daily. All animals in this investigation received 6 drops of cod liver oil, as a source of vitamins A and D, daily except Sundays.

The animals at initial weights of 40 to 70 gm. were taken from our stock colony which is fed our diet no. 2, the one introduced by Sherman and Muhlfield ('22). The fact that the vitamin deficient animals were able to make slow but continuous growth on ration 10 would indicate that the 80% alcoholic extracts from rice polishings provide appreciable amounts of riboflavin. Five pathological animals, however, out of eighteen died from riboflavin deficiency while subsisting on this ration. Fortunately the collapse occurred in the daytime so the final weight records are accurate. In the case of the animals on rations 11 and 12, sufficient riboflavin

for slow growth of the pathological rats must have been furnished by the "W" factor extracts, although such extracts were treated twice with fuller's earth at a pH of 3 to 4. The marked differences, however, in growth between the pathological and litter mate controls that received 20 μg. riboflavin daily are evident from tables 1 and 2.

At the termination of the experiments twenty pairs of rats selected from series I and II, were freed from their alimentary fills, ground through a sausage mill and dried to constant weight in an electric oven at a temperature of about 55°C. They were then extracted with ether in continuous Soxlet extractors for 24 hours. The ground fat-extracted rats were then reground in the sausage mill to a fine powder and redried at 103°C. for several hours during which procedure the small amounts of residual moisture were lost. One gram samples in duplicate were then taken for ash and total nitrogen determinations. The total nitrogen obtained multiplied by the factor 6.25 was taken as the protein in the sample. In addition, twenty-six rats from the same rat colony as the experimental animals were taken for analysis. Half of these were males and half females, with weights and ages the same as those of the animals of series I and II when they were started on experiments. The body carcasses of these animals were also freed from alimentary fills, dried and analyzed for fat, ash, and protein. The differences in chemical composition of the dry carcasses of these rats and the dry carcasses of the animals sacrificed at the termination of the experiments were taken as representing the fat, ash, and protein contents of the body gains. All the results of this investigation are presented in tables 1 and 2.

The experiments of series I (table 1) were performed on a total of eighteen pairs of animals, fourteen of which were male, and four female. The period of experimentation ranged from 50 to 188 days. The animals were given ration 10 supplemented with pyridoxin and thiamine as previously indicated. The "W" factor given in three cases for 16 to 34 days

was not found essential as a supplement of the vitamin B complex and was soon omitted.

The control animals that received the same ration and the same amount of food as the pathological rats, but in addition 20 μg. riboflavin daily, showed increased gains of 56 to 1300% over their riboflavin deficient litter mates. Although the period of experimentation differed for the various pairs of rats, the treatment of the control rats was in every case the same as that of their respective litter mates except for the fact that the former received riboflavin supplements. It, therefore, seems justifiable to present average gains per animal in this as well as the second series. In series I the average gain in body weight per animal was 43.8 gm., and for the control animals 82.2 gm. The pronounced influence of riboflavin in economy of food utilization for synthesis of body tissue is then most evident.

In series II there were six pairs of females and nine pairs of males maintained on rations 11 and 12, in which the components of the vitamin B complex with the exception of the filtrate factors were furnished in the form of crystalline vitamins. The filtrate factors were supplied by the "W" extracts prepared according to the procedure of Frost and Elvehjem ('37). The results in this series are also consistent, fourteen out of the fifteen pairs showing larger gains in weight in the control animals compared with the riboflavin deficient litter mates. The average gain in weight per animal for the riboflavin deficient animals was 56.0 gm. and for the litter mate controls on the same ration and same amount of food, 91.0 gm. Since the results of series II experiments are very similar to those of series I, detailed data are not presented.

The influence of riboflavin deficiency on chemical composition of the bodies was determined on twenty pairs of animals, but to conserve space, such figures are omitted. These figures were, however, used in calculations of chemical composition of body gains which are presented in table 2. There were no noteworthy differences in moisture content between the ribo-

TABLE 1

The role of riboflavin in economy of food utilization (series I, ration 10)

PAIR NUMBER [1]	PERIOD OF EXPERIMENTATION	BODY WEIGHT		CHANGE IN WEIGHT
		Initial	Final	
	days	*gm.*	*gm.*	*gm.*
1 — P [2]	94	53	90	+37
RC	94	50	146	+96
2 — P	50	44	51	+7
RC	50	47	68	+21
3 — P	122	39	100	+61
RC	122	38	133	+95
4 — P	101	40	56	+16
RC	101	38	91	+53
5 — P	108	38	68	+30
RC	108	38	96	+58
6 — P	79	40	78	+38
RC	79	40	100	+60
7 — P	155	38	147	+109
RC	155	38	188	+150
8 — P	156	38	82	+44
RC	156	38	154	+116
9 — P	98	36	64	+28
RC	98	38	114	+76
10 — P [2]	72	38	36	—2
RC	72	38	55	+17
11 — P [2]	50	34	36	+2
RC	50	37	63	+26
12 — P	77	40	38	—2
RC	77	40	66	+26
13 — P	78	49	66	+17
RC	78	49	108	+59
14 — P [2]	72	44	54	+10
RC	72	38	67	+29
15 — P	181	38	162	+124
RC	181	38	233	+195
16 — P [2]	78	52	57	+5
RC	78	53	78	+25
17 — P	188	48	165	+117
RC	188	47	228	+181
18 — P	185	44	192	+148
RC	185	44	240	+196

[1] P = pathological; RC = restricted control.

[2] Animal died.

flavin deficient and control rats. From table 2 it is apparent that the greatest difference in chemical composition of body gains between the riboflavin deficient and the control rats was in fat, the control rats having gained eighty to several hundred

times more in fat than the litter mate pathological animals. The control rats also showed considerably more gain in protein than the pathological animals but the changes in ash are too small and variable to be of any significance.

TABLE 2

Influence of riboflavin deficiency on changes in chemical composition of body gains

RATION NUMBER	PAIR NUMBER [1]	PERIOD OF EXPERIMENTATION	CHANGE IN BODY WEIGHT	CHEMICAL COMPOSITION OF BODY GAIN		
				Fat	Ash	Protein
		days	*gm.*	*gm.*	*gm.*	*gm.*
11	21 — P	92	+34	—3.17	+2.59	+9.95
	RC		+79	+3.99	+4.53	+20.28
11	19 — P	135	+79	+9.56	+3.32	+16.80
	RC		+103	+14.42	+6.12	+25.36
11	26 — P	136	+61	+7.82	+3.53	+12.41
	RC		+98	+9.88	+4.67	+20.94
11	28 — P	127	+88	+7.11	+4.79	+19.64
	RC		+141	+13.31	+5.63	+30.98
11	23 — P	80	+12	—3.85	+2.43	+5.11
	RC		+52	—0.40	+3.16	+14.66
11	25 — P	72	+31	+2.69	+1.93	+11.47
	RC		+38	+4.88	+1.79	+11.91
11	29 — P	179	+50	+1.44	+3.82	+13.32
	RC		+80	+6.33	+4.24	+18.93
11	32 — P	219	+74	+4.49	+4.23	+16.41
	RC		+132	+22.72	+5.59	+25.35
12	31 — P	153	+94	+3.64	+5.35	+21.90
	RC		+134	+21.57	+6.02	+28.91
11	33 — P	212	+120	+7.88	+6.68	+24.07
	RC		+153	+16.17	+8.57	+30.13
10	2 — P	50	+7	—2.86	+1.45	+3.90
	RC		+21	+0.43	+1.28	+6.11
10	9 — P	98	+28	—3.43	+3.01	+5.08
	RC		+76	+3.85	+2.66	+13.97
10	7 — P	156	+44	+0.47	+3.20	+12.00
	RC		+116	+19.40	+5.37	+30.21
10	11 — P	72	+2	—3.34	+0.78	+0.71
	RC		+26	+0.35	+1.64	+7.25
10	13 — P	78	+17	—3.56	+2.03	+5.03
	RC		+59	+4.24	+3.25	+15.98
10	15 — P	181	+124	+10.33	+5.69	+23.80
	RC		+195	+20.25	+8.44	+45.58
10	17 — P	188	+117	+8.61	+6.27	+23.38
	RC		+181	+30.28	+7.62	+36.06
10	18 — P	185	+148	+7.82	+7.37	+39.21
	RC		+196	+36.12	+8.27	+37.57
10	6 — P	79	+38	+2.25	+1.49	+6.45
	RC		+60	+3.74	+4.05	+14.40
10	4 — P	101	+16	—2.18	+1.60	+4.30
	RC		+53	+0.27	+3.06	+12.62

[1] P = pathological; RC = restricted control.

DISCUSSION

The only investigation that approximates ours reported in the literature is the one by Braman and associates ('35). These investigators reported on twelve pairs of rats studied by the paired-feeding method and subsisting on diets deficient in vitamin G for a period of 14 weeks. Considerable difficulties were experienced in keeping their vitamin G deficient animals alive and frequently small amounts of yeast were given. The fact that the pathological rats developed dermatitis would indicate that there was also a deficiency of the acrodynia factor, pyridoxin. The control animals received an aqueous extract of Lilly's liver concentrate no. 343, as a source of vitamin G following the seventh week; previously dry-heated yeast was used. The average gain in body weight per animal was 25.9 gm. for the vitamin G deficient animals and 41.3 gm. gain for the controls, or an increase in 59.4% representing economy of food utilization for the animals receiving the vitamin G supplement. The chemical composition of the body gains showed 0.6 gm. of fat and 1.22 gm. nitrogen for the pathological animals and 3.1 gm. of fat and 1.74 gm. nitrogen for the controls. It is interesting to observe that, although these investigators were handicapped in 1935 in the construction of an uncomplicated riboflavin deficient diet, the relative differences they found in gains of body fat in their vitamin G deficient and control animals compare strikingly with the differences in gains of fat found in our riboflavin deficient and control animals. The authors, however, state: "That liver contains an abundance of vitamin G has been quite established, and there is evidence that it may also contain other factors especially favorable to growth and reproduction. It is recognized, therefore, that the water extract of the liver concentrate used in this experiment may have furnished more than a single factor in exerting its influence on the utilization of the protein and energy of the food." On the other hand, from the results of our investigation, it is quite clear that we were concerned with only one limiting factor, namely, riboflavin deficiency.

SUMMARY

Riboflavin produces a most pronounced effect on economy of food utilization for synthesis of body tissues. The increases in body gains were derived mainly from fats and to a lesser but appreciable extent from proteins.

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VITAMIN A DEFICIENCY: A FIELD STUDY IN NEWFOUNDLAND AND LABRADOR

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ONE FIGURE

(Received for publication September 25, 1940)

For the past 3 years this laboratory, with others, has studied the course and cure of night-blindness in experimental vitamin A deficiency (Wald, Jeghers and Arminio, '38; Wald and Steven, '39). In order to apply and extend this work, we undertook a field study in the region of Northern Newfoundland and Labrador served by the International Grenfell Mission. Beriberi is endemic in this area, and recurrent epidemics of night-blindness have been reported from it (Appleton, '21; Aykroyd, '30). The measurements were made by one of us (D. S.) from September, 1939, through May, 1940. They are concerned with the design and application of a visual method for the survey of vitamin A deficiency; and with a study of the mutual effects of vitamin A deficiency and beriberi upon the visual threshold.

I. THE MEASUREMENT OF VITAMIN A DEFICIENCY

A. Apparatus and procedure

For the measurement of visual threshold a portable adaptometer was designed by one of us (G. W.).[2] It weighs about 20 pounds, and is completely enclosed in a cabinet 8.5 × 8.5 ×

[1] This research has been aided in part by grants to D. S. by the Sir Charles and Lady Julia Henry Fund, and the Permanent Science Fund of the American Academy of Arts and Sciences; and by grants to G. W. by the Milbank Fund and the Josiah Macy, Jr. Foundation.

[2] A detailed description of this instrument has appeared in the Journal of the Optical Society of America, 1941, vol. 31, p. 235.

11.5 inches in size. It includes a test unit, resembling a pocket lamp in size and shape, which contains the test lamp, a shutter set at 1/50 second, and at its front the test field. On top of this unit a small housing containing a miniature lamp provides a red fixation point. A funnel-shaped eye-piece which attaches to the test unit sets the subject's head in the correct position and protects the eyes from stray light.

The test unit is connected with a long flexible cord to the cabinet, which contains storage cells, a voltmeter and rheostat for regulating the line voltage, and a calibrated rheostat with scale for controlling the brightness of the test patch. This can be varied over an effective range of about 1 to 1 million. It was calibrated in milli-microlamberts (10^{-6} millilamberts) with a Macbeth illuminometer. This instrument measures brightness for the human eye directly, and so circumvents the difficulty that a rheostat modifies the color of the source as well as its intensity. All brightnesses in the present paper are expressed logarithmically for reasons which have been discussed previously (Wald et al., '38).

The subject faces a circular test field which subtends an angle of 2.4 degrees with the eye, and which is centered 6 degrees below the fixation point. In performing a measurement the test field is set initially at a sub-threshold intensity. Between successive flashes the intensity is raised by roughly equal logarithmic steps (i.e., by a constant proportion), until the subject reports just seeing the flash. The scale reading and time in darkness are then noted.

Following the recommendation of Wald et al. ('38) we measured only the threshold of the completely dark adapted eye. This rises earlier and further in vitamin A deficiency than the threshold in any other state of adaptation. It maintains a constant level at each sitting and so permits repetition and averaging of the measurements. It is probably the most accurate single index of vitamin A deficiency now available.

Readings were made after a subject had been in complete darkness for at least 20 minutes, and when necessary were

continued until a constant level was attained. Usually several persons were dark adapted simultaneously. As each subject left the dark room another entered. In this way, following the initial 20-minute interval, measurements could be continued without interruption. Tests on each individual were usually completed within 5 minutes.

Reliable measurements could not be obtained from children below 6 years of age. In the age group 6–8 years about one-quarter of the subjects had to be discarded.

B. The "normal range"

In the initial period of the research fifty children from the Orphanage and seventy-eight children from the Mission School in St. Anthony were tested. Both groups ranged in age from 6 to 17.

The Orphanage children received daily supplements of cod liver oil containing about 15,000 International Units (I.U.)[3] of vitamin A, or two and one-half times the highest estimates of optimal requirement. These children therefore represent a reliable normal control. The distribution of their visual thresholds is shown in figure 1. The mean log threshold is 2.17 and the spread about 1 logarithmic unit. Similar distributions of normal thresholds have been reported by Lindqvist ('38) and by Hecht and Mandelbaum ('39).

The Mission School children lived at home and ordinarily received no vitamin supplements. Nevertheless their threshold distribution, also shown in figure 1, was very similar to that of the Orphanage group. The mean log threshold in this instance was 2.25.

The impression is widespread that such a "normal range" of thresholds might provide the primary or even sole basis for the diagnosis of vitamin A deficiency. Actually it is

[3] Throughout this paper all vitamin A concentrations are expressed in the International Unit (I.U.), equivalent to one U.S.P. XI Unit, or to 0.6 μg. of pure β-carotene. Concentrations of thiamine (vitamin B_1) are expressed in International Units (U.S.P. XI), each of which is equivalent to the vitamin B_1 activity of 10 mg. of the International Standard adsorbate.

entirely inadequate for this purpose. High visual thresholds may result from a variety of congenital and acquired abnormalities other than deficiency in vitamin A. Conversely there is latitude enough for a threshold to rise considerably above its optimal value and still remain within the "normal range".[4]

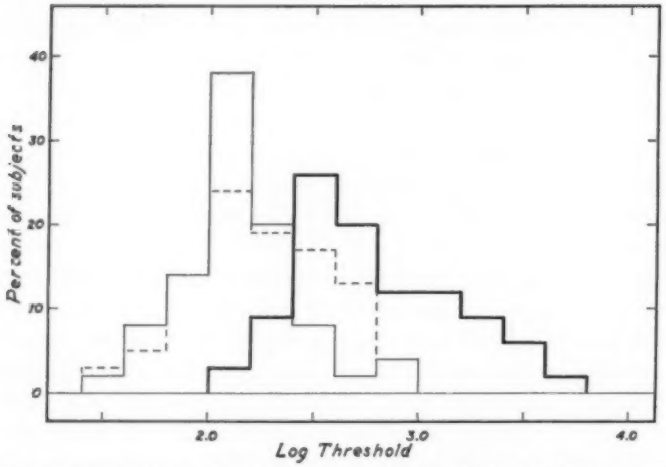

Fig. 1 Distribution of visual thresholds of fifty children optimally supplied with vitamin A (fine line); of seventy-eight unselected school children (broken line); and of thirty-four vitamin A deficient subjects (heavy line).

The threshold distribution of our vitamin A deficient subjects, as shown in figure 1, overlaps widely with the control distribution. About half of the vitamin A deficient cases possessed log thresholds well within the "normal range". Conversely, of those subjects who possessed log thresholds above 2.7, only half proved to be vitamin A deficient. Clearly

[4] *Case 1.* Mrs. R., 38. Pregnant 8 months. Had had seal meat about twice weekly, brown flour, some butter; but no milk, fresh vegetables or game for months. Initial log threshold, 2.49. Three and one-half hours after administration of 100,000 I.U. of carotene her log threshold had fallen to 1.67. After 3 days supplementation with 10,000 I.U. of carotene the log threshold was virtually unchanged, 1.76. This patient's threshold had been displaced from the top to the bottom of the "normal range" by carotene administration.

a more searching criterion than low or high threshold is needed to estimate vitamin A deficiency.

C. The vitamin A-labile threshold

A subject may be regarded as vitamin A deficient when his visual threshold, whatever its initial level, is lowered significantly by administration of vitamin A, or is vitamin A-labile. We do not yet know that this definition is exhaustive, but it probably comes as close to the root of the matter as is at present possible.

Repeated measurements by our methods have shown that the threshold of a normal subject generally varies during one sitting within a range of about 0.1 log unit, and varies from day to day within a range of about 0.3 log unit, or about 100%. Only changes which transcend these limits, therefore, are significant in the present procedure.

Vitamin A-lability was determined as follows. The visual threshold of a subject having been measured, a known dose of a vitamin A preparation was given orally. Usually within several hours the threshold was redetermined. A decrease in threshold of at least 0.3 log unit, or eventual lowering by this amount over a longer period of supplementation, was accepted as evidence of vitamin A deficiency.

In general a threshold which reacted to a single dose of the vitamin did so within 1 to 6 hours. In some instances, however, significant changes were noted only after 24 hours to 48 hours, and additional supplementation. Usually if the first dose of vitamin A yielded no significant result, further doses were given and tests conducted over as long a period as the patient remained available. A subject whose threshold was not significantly lowered by at least 2 weeks of regular supplementation was regarded as non-deficient.

Originally vitamin A was given only to those subjects with log thresholds above 2.7. When this procedure had proved unreliable, the vitamin was given to all subjects with thresholds in the upper portion of the "normal range", and, re-

gardless of this, to all those with beriberi symptoms or poor dietary histories. Ideally, the thresholds of all subjects should have been tested for vitamin A-lability.

II. THE INCIDENCE AND DEGREE OF VITAMIN A DEFICIENCY

During the period of the survey, 353 persons were examined. Their origins are shown in table 1, A. The fifty-eight Orphanage children form a distinct control group. Fifteen patients hospitalized or receiving outpatient care for beriberi constitute a group specially selected for nutritional disease.

TABLE 1

A. Distribution of persons tested

St. Anthony Orphanage	58
St. Anthony Mission School	82
Cartwright Mission School	51
St. Anthony Hospital and Outpatients	103
At home	59
Total	353

B. Vitamin A deficiency and beriberi

	CASES	AVERAGE AGES	MALES: FEMALES
Vitamin A deficiency alone	19	26.2	7:12
Beriberi alone[1]	17	39.6	13:4
Vitamin A deficiency and beriberi	15	41.1	13:2
Total vitamin A deficient	34	32.8	20:14
Total with beriberi	32	40.3	26:6

[1] This group includes a number of cases with thresholds in the "normal range" but not completely tested for vitamin A-lability.

The remaining 280 subjects probably represent a fair sample of the general population. They include eighty-eight patients under hospital treatment for other causes than nutritional deficiency; and seventeen subjects found at large with beriberi, in apparent accord with the random distribution of such cases.

Vitamin A-labile thresholds were found in thirty-four subjects, or 9.7% of the total number examined (table 1, B). Of these, seven had been hospitalized for beriberi. The incidence of vitamin A deficiency among the "random" subjects, therefore, was 27 out of 280, or still 9.7%.

Most of these subjects exhibited low degrees of deficiency. About half of them possessed thresholds within the "normal range." At most nine subjects, or 3% of all those examined, might have been described as clinically night-blind. Only two complained of poor vision in dim light. No xerophthalmia or keratomalacia was encountered. We conclude that severe vitamin A deficiency rarely occurs in this population at present.

This situation is closely connected with the time required for night-blindness to develop. On experimentally A-deficient diets the visual threshold in some cases rises at once, in others only after several weeks or months (Wald and Steven, '39; Hecht and Mandelbaum, '40). The latter result appears at present to be the more usual. We have now encountered it frequently in the field. A considerable number of our subjects had subsisted for weeks or months exclusively on the regional staples, white bread, tea, and molasses or sugar. Almost all such persons displayed symptoms of beriberi, sometimes severe. Yet regularly they possessed visual thresholds well within the "normal range." [5] This prolonged resistance to vitamin A deficiency undoubtedly accounts in large part for the low incidence actually encountered.

About 5% of all the subjects examined possessed stable visual thresholds and good dietary histories and were almost certainly not vitamin A deficient, yet were highly night-blind.[6]

[5] *Case 2.* E. P., female, 34. Beriberi following childbirth. Legs numb, no patellar reflex, anaesthetic to pin-prick. Unable to walk. Tightness in epigastric region, dyspnoea, edema before delivery. Had given birth to twins 44 days previously, of which one died on the way to the hospital, the other shortly after admission. Log threshold, 2.00.

[6] *Case 3.* E. M., 13, female. Cartwright Mission School. A normal, well-fed, healthy-looking girl. She had received daily supplements of cod liver oil since arriving at school more than 2 weeks before. Her log threshold averaged 3.80, or about 1 log unit above the upper limit of the "normal range". She was given about 50,000 units of vitamin A. After 2½ hours, her log threshold averaged 3.89. She was re-tested on the following day: log threshold, 3.71. The vitamin A had had no appreciable effect. This subject is markedly night-blind with no evidence whatever of vitamin A or other nutritional deficiency.

This proportion appears to be extraordinarily high, and may be due to the spread of a hereditary factor for night-blindness within this unusually inbred population.

III. VITAMIN A DEFICIENCY AND BERIBERI

Beriberi is endemic in Northern Newfoundland. The number of hospital admissions is only a crude index of its incidence. In general only severe cases are admitted, in which the limbs are paralyzed or the heart dilated. Symptoms of less severe deficiency are widespread among the population. A syndrome known locally as "Newfoundland stomach" is probably at least in part due to mild thiamine deficiency; its symptoms are prolonged constipation and a number of vague general complaints, such as irritability, dyspepsia, itching and burning sensations, and lassitude. Doctor La Salle of Port Saunders, Newfoundland, has treated this ailment with thiamine chloride and in some instances observed almost immediate relief of symptoms (personal communication).

In the succeeding discussion the term beriberi refers only to the acute disease, characterized by clinically clear neural or cardio-vascular disturbances. In all, thirty-two such cases, fifteen of which were sufficiently severe to require hospitalization, were tested for vitamin A deficiency. The results are listed in table 1, B.

Fifteen of the beriberi subjects possessed definitely vitamin A-labile thresholds. All the others possessed thresholds which were either stable to vitamin A, or well within the normal range. Some of the latter were not exhaustively tested for vitamin A-lability, and may possibly have included several incipient A-deficient individuals. In any event the incidence of vitamin A deficiency in the beriberi group is about five times as high as in the general population. This is not surprising, since the highly restricted diets common in this region are undoubtedly deficient in many factors simultaneously.

Beyond this, however, no intrinsic relation appears to exist between mild vitamin A deficiency and beriberi.[7] Several subjects with severe beriberi exhibited normal visual thresholds, stable to vitamin A (cf. case 2, above).[8] In patients with both deficiencies, administration of brewer's yeast alone did not lower the visual threshold significantly, while vitamin A alone was as effective as vitamin A and brewer's yeast together.[9]

Vitamin A usually lowered the thresholds of such subjects to well within the normal range. There is therefore no reason to believe that even a residue of night-blindness is due to beriberi itself.

IV. CORRELATIONS

Age and sex. (Table 1, B.) Both vitamin A and beriberi—excluding infantile beriberi with which we did not deal—are diseases of maturity in the region of this survey. Though more than half of all our subjects were school children, none

[7] *Case 4.* R. H. C., 54, male. Moderate symptoms of beriberi; log threshold, 3.6. Within 1½ hours after administration of 40,000 I.U. of carotene his threshold had fallen 0.75 log unit, or to about ⅛ its initial value. He was left some carotene and a small supply of yeast. Three weeks later he was admitted to hospital with more severe symptoms of beriberi. His visual threshold, however, had fallen still further, to 2.55. On regular daily administration of 25,000 units of vitamin A, the log threshold sank within 4 days to 1.9. It remained at this level until the patient's discharge 20 days later. This case demonstrates a very large improvement in visual threshold coincident with intensification of beriberi symptoms.

[8] *Case 5.* L. C., male, 28. Beriberi. Legs weak and at times edematous, hyperaesthetic to pin-prick. Tightness in epigastric region; dyspnoea. Could work only for short intervals. His diet throughout the winter had been white bread and tea; no meat or vegetables, negligible butter. Log threshold, 2.47. After taking 110,000 I.U. of vitamin A over a 3-day period, his log threshold was 2.30. This is not a significant change.

[9] *Case 6.* J. P., 41, male. Mild beriberi; initial log threshold, 2.95. Twenty-four hours following administration of brewer's yeast containing abouf 500 I.U. of thiamine the threshold had not significantly changed (2.8). One and one-half hours after administration of 50,000 I.U. of carotene the log threshold had fallen to 2.45, and on re-test 24 hours later had fallen to 2.25.

of the beriberi cases and only three of the vitamin A deficiencies were in the age range 6 to 15. Moreover, the vitamin A deficient subjects were in general much younger than those with beriberi. The average age of the former group was 26.2; or, with two exceptionally old male subjects excluded, 22.3. The average age of the beriberi cases was almost twice as great, 40.3.

These disorders also affect the sexes differentially. A small, perhaps not significant majority of the vitamin A deficient subjects were males. About 4/5 of the beriberi cases, however, were men; and half of the latter were simultaneously vitamin A deficient.

Season. Seasonal epidemics of night-blindness have been reported from the area of this survey (Appleton, '21; Aykroyd, '30). No epidemic was observed during the period of this research, nor did inquiry reveal any general incidence of night-blindness in this population in recent years. The visual thresholds of fourteen children at the St. Anthony Mission School were measured repeatedly during the entire course of the research, but they displayed no appreciable seasonal fluctuation.

Beriberi, however, attained a high peak in February, with seven cases, and in March, with sixteen. Nine of the March cases were simultaneously vitamin A deficient. These months, in which winter stores are running low and new supplies have not yet become available, represent the nutritional low of the year.

DISCUSSION

The response to vitamin A. The usefulness of our method for estimating vitamin A deficiency depends in part upon the speed with which the night-blind retina reacts to vitamin A. Some question has recently arisen concerning this matter. The following summary may clarify its present status.

1. Clinical studies of acute dietary night-blindness now embrace well over 1000 cases (Kubli, 1887, 320 cases; Birn-

bacher, '27, 330 cases; Bloch, '20–'21; Appleton, '21; Aykroyd, '30; Mori, '32). All these workers agree that nearly always the subjective and crude objective symptoms of night-blindness vanish within a few hours to a few days after administering a complete diet or vitamin A supplements. Judging by laboratory experience, the decreases in threshold which accompany such cures could not well be less than a full logarithmic unit.

2. Birnbacher's study included crude measurements of the visual threshold. He observed that though several meals containing roast mammalian liver almost invariably dissipated all clinical signs of night-blindness, in about half the cases the visual threshold continued to fall through a further period of supplementation. He rarely encountered patients in whom complete cures took longer than 4 weeks; though in a few extreme cases it occupied several months. It must be noted that by present standards Birnbacher's supplementation was very low. Our own observations are consistent with this report.

3. A few carefully controlled studies of experimental night-blindness have been recorded. Jeghers ('37 b), having produced mild night-blindness in himself, observed a significant improvement within 2 hours and complete cure within 3 days of heavy dosage with vitamin A. Wald et al. ('38, '39) repeatedly obtained complete reversals of mild night-blindness in two subjects within 1½ to 2½ hours after large oral doses of vitamin A or carotene. Lewis and Haig ('39) obtained similar results with three young children. Booher et al. ('39) found minimal doses of cod liver oil to take effect in five subjects within 2–3 hours after administration.

Hecht and Mandelbaum ('40), however, report that the complete reversal of night-blindness in all nine of their subjects required from 6 weeks to over 3 months of heavy vitamin A supplementation. Their results are unique; not because the observed responses were slow, but because they were invariably so. The consensus of all the clinical and most

other laboratory reports shows this slow type of response to be relatively rare.[10]

Hecht and Mandelbaum's charts as drawn suggest another peculiarity—occasional rise of visual threshold during heavy feeding with vitamin A concentrates. It may be hoped that these changes are not significant, since they fall within the authors' stated range of day-to-day variation.

4. Our criterion for vitamin A deficiency is a decrease in threshold of at least 0.3 log unit within at least 2 weeks of regular vitamin A supplementation. As an extreme test, this may be appraised in the light of Hecht and Mandelbaum's results. In six out of nine of their subjects tested with single large doses of vitamin A, the visual threshold decreased by 0.3 to 1.0 log unit within 3–8 hours. In two further subjects, the threshold fell by this amount within about a week of supplementation. The final subject required about 4 weeks for significant change. Another subject who had not been tested with single large doses responded still more slowly. In this extreme situation, therefore, our method, strictly applied, might have overlooked two out of these ten deficiencies.

From all the information now available it may be concluded that an undetermined but in any case small percentage of vitamin A deficient subjects might fail to respond within 2 weeks of regular supplementation. It has not yet been shown that this occurs to a significant degree under field conditions. Even this potential disability in the procedure, however, may be corrected by lengthening the period of supplementation in doubtful cases.

[10] Hecht and Mandelbaum cite in support of these observations the experiments of Steffens, Bair and Sheard ('39) and of McDonald and Adler ('40). The first reference is clearly in error; as the authors state, their three subjects failed to respond to vitamin A because they were not night blind. The second paper concerns a single subject whose threshold on an experimental diet rose about 1 log unit in 4 weeks, then took over 8 months of normal and supplemented diets to return to normal. It is extraordinary, not to obtain this result once, but to obtain it exclusively, as have Hecht and Mandelbaum. It is still entirely possible that this is a laboratory artefact, associated with certain conditions surrounding the production of experimental night-blindness.

Incidence of vitamin A deficiency. A number of recent researches purport to show that mild night-blindness is widespread within the general populations of European countries and the United States (cf. reviews by Jeghers, '37 a; Lindqvist, '38). These results were obtained with frankly empirical, arbitrary methods, the physiological meaning of which is obscure.

One procedure, developed by Jeans et al. ('36, '37) using the Birch-Hirschfeld Photometer or the Biophotometer, has revealed "abnormal" thresholds believed to be due to vitamin A deficiency in about 20–80% of various groups of American and English school children and adults. The Edmund test, based upon brightness discrimination and in wide use in the Scandinavian countries, has yielded at times even higher deficiency estimates. But Palmer and Blumberg ('37) and Isaacs, Jung and Ivy ('38) have failed to confirm these results with Jeans's methods and have seriously questioned their reliability. Lindqvist ('38) and Groth-Petersen ('38) have similarly criticised the Edmund procedure. Both methods as originally employed apparently suffered from an uncritical appraisal of the variability of the measurements, and of the limitations of a "normal range".

Another group of methods has employed the threshold of the dark adapted eye in some context which makes the procedure specific—for example, the determination of vitamin A lability or of blood vitamin A. Such methods have so far indicated a relatively low incidence of vitamin A deficiency. Lindqvist found abnormally high visual thresholds accompanied by abnormally low serum vitamin A concentrations in less than 5% of a group of hospital patients particularly suspected of vitamin A deficiency. Lewis and Haig ('40) found only one abnormally high and vitamin A-labile threshold among 144 children, mostly from poor homes, under hospital care in New York. The present survey, conducted upon a population chosen for its long history of deficiency diseases, reveals an incidence of vitamin A-labile thresholds of about 10%, more than half of them within the "normal range".

Probably more extensive use of controlled and accurate procedures will show that even very mild vitamin A deficiency ordinarily is rare in occidental populations.

We are greatly indebted to Dr. Charles Curtis, director of the International Grenfell Association Mission, and to its staff, for the many courtesies and facilities which made this research possible.

We are indebted also to the Abbott Laboratories of North Chicago for the halibut liver oil capsules and brewer's yeast tablets; and to the S.M.A. Corporation of Chicago for capsules of carotene in oil, used as supplements in this research.

SUMMARY

1. The incidence of vitamin A deficiency was surveyed by visual methods in an area from which epidemic night-blindness has been reported. With a specially designed portable adaptometer, the threshold of the completely dark adapted eye was measured. Rise of this threshold above a "normal range" proved inadequate as an index of deficiency. Deficiency is indicated reliably by a vitamin A-labile threshold—one that, whatever its initial level, is lowered at least 0.3 logarithmic unit within 2 weeks of regular vitamin A supplementation. In practice a threshold which reacted to vitamin A usually did so within 1–6 hours.

2. Vitamin A-labile thresholds were found in 9.7%, and clinical night-blindness in at most 3% of the subjects. About half the A-labile thresholds were within the "normal range". About half were found in subjects suffering also from beriberi.

3. Vitamin A-labile thresholds and beriberi are mutually independent. Acute beriberi may be accompanied by normal thresholds, stable to vitamin A. In subjects with both deficiencies, brewer's yeast does not lower the threshold, while vitamin A alone is as effective as vitamin A and brewer's yeast together.

4. Both vitamin A deficiency and beriberi are most prevalent in February and March. Both (infants excluded) are found

principally in adults. Vitamin A deficiency occurs in both sexes at average age about 26, beriberi primarily in males of average age about 40.

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DEPLETION OF TISSUE GLYCOGEN DURING FASTING AND FATIGUE AND PARTIAL RECOVERY WITHOUT FOOD

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This study was made for the purpose of securing base-line values from which to calculate the recovery of glycogen at intervals of 1, 2 and 3 hours following fatigue and feeding with known amounts of glucose and fructose (see following paper: Nutter and Murlin, '41). A considerable amount of time also was spent in evaluation of the analytical methods for determination both of glucose and glycogen which, from the literature, appeared to be most appropriate to the purpose. Results of the methodological work will be described briefly first.

In control experiments with dry C. P. glucose the average recovery was 99.67% by the copper-iodometric method of Shaffer and Somogyi ('33); 100.54% by direct iodometric oxidation as described by Bierry, Gouzon and Magnan ('36); and 104.57% by Bierry and Goiran's ('28) micro modification of Bertrand's ('06) cupric procedure. With the last-named method great difficulty was experienced in reaching an accurate end point, and wide variations in results were obtained. Since the later method of Bierry et al. ('36) offered no advantage, the Shaffer-Somogyi method was adopted.

The effect of various factors upon glycogen recovery was investigated thoroughly, and as a result the method of Good, Kramer and Somogyi ('33) was adopted after modification

[1] Taken from a thesis submitted in partial fulfillment of the requirement for the Doctorate of Philosophy, University of Rochester, June, 1938.

as follows: Tissues were dissolved in 45 instead of 30% KOH, glycogen was precipitated by addition of 3 instead of 1.1 volumes of 95% alcohol, and this was followed by a second precipitation in the same manner. Recovery of added dry "C. P." glycogen [2] on the basis of the actual glycogen was 99.19%.

PROCEDURE

Young adult albino rats, falling within a restricted range of weight (averaging about 140 to 150 gm.), and nearly all of the female sex, were selected from the stock colony. They had been maintained previously on a uniform diet.[3] Food was removed from the cages 24 hours prior to the beginning of the experimental run, but the animals were allowed drinking water ad libitum. To avoid, so far as possible, the variations due to diurnal changes in glycogen storage observed by Forsgren ('28) as well as by Ågren, Wilander and Jorpes ('31), the experiments were arranged so that the tissue samples would be taken at a fairly uniform time, namely, late in the afternoon.

Attempts to fatigue the rats by voluntary exercise in revolving cages were not successful, due to wide individual variations in inclination for this kind of work. Neither the sight of food nor exposure to cold or darkness, or both, stimulated any greater activity. Later trials with a treadmill were also unsatisfactory, since, with a few exceptions, even repeated electrical stimuli were ineffective in forcing the animals to run. In the experiments herein reported, the rats, fasted for 24 hours prior to the experiment, were fatigued by swimming in water at 30°C. until exhausted [4] (a period of some 8 to 11 hours), after which they were dried and placed in warm cages.

[2] Pfanstiehl.

[3] Purina Dog Chow.

[4] Non-fatigued animals, when removed from the swimming tank, run about the floor in normal fashion. The exhausted animals show little inclination to move and apparently lack powers of coordination so that they stagger about in a drunken manner.

In order to establish a basal level of glycogen, fasted and fatigued controls were allowed to recover, without carbohydrate or other food, for 1, 2 and 3 hours. In preliminary experiments the animals were prepared for the removal of tissue samples by stunning them with a blow on the head. This method was abandoned because of the frequently accompanying convulsive movements, with consequent glycogenolysis in the muscles. Careful administration of sodium amytal obviated any struggling in the animal.

Therefore, at the end of the recovery period, the animals were anaesthetized by intraperitoneal injection of 10% sodium amytal, prepared according to the method of Mulinos ('28), the dosage being 10 mg. per 100 gm. of female rat and 20 mg. per 100 gm. of male rat (Nicholas and Barron, '32). After anaesthesia was established, the abdomen was opened and a lobe of liver tissue was rapidly excised. The blood was soaked up in filter paper and the sample was immediately dropped into a tared tube of warm 45% KOH solution. The gastrocnemius muscle was then extirpated rapidly, with precautions to avoid twitching or damaging of the fibers, and was also dropped into KOH. The vastus lateralis muscle was used in a very few of the earlier experiments, but it was found that the gastrocnemius could be dissected out more quickly and without cutting into the muscle fibers. After determination of their weights, the tissue samples were dissolved by digestion for about ½ hour in a boiling water bath with frequent shaking to hasten the disintegration.

EXPERIMENTAL RESULTS

According to the usual custom, all tissue glycogen values are calculated in terms of milligrams of glucose, but expressed as glycogen. Table 1, containing representative examples of the values obtained for the glycogen content of paired muscles, shows that large differences (0.36% to 38.45%) occurred after stunning the animal, whereas closely agreeing results (varying from 0.06% to 0.67%) were obtained under amytal anaesthesia. It is interesting to note that, in the animals

TABLE 1

Glycogen content of opposite paired muscles after stunning and under amytal anaesthesia

RAT	BODY WEIGHT	METHOD OF TREATMENT	GLYCOGEN		DIFFERENCE	DIFFERENCE
			In right muscle	In left muscle		
	gm.		*mg.%*	*mg.%*		%
7	220	Killed by stunning	409.4	316.8	92.6	22.62
18	115	Killed by stunning	208.6	205.4	3.2	1.53
20	135	Killed by stunning	141.0	141.1	0.1	0.71
27	150	Killed by stunning	375.3	346.5	28.8	7.67
38	149	Killed by stunning	332.5	329.5	3.0	0.90
37	135	Killed by stunning	304.7	254.2	50.5	16.57
9	185	Killed by stunning	174.5	107.4	67.1	38.45
51	197	Killed by stunning	333.1	331.9	1.2	0.36
56	182	Anaesthetized by amytal	349.6	349.3	0.3	0.09
58	215	Anaesthetized by amytal	605.8	609.9	4.1	0.67
69	206	Anaesthetized by amytal	348.1	347.9	0.2	0.06
93	144	Anaesthetized by amytal	164.2	164.6	0.4	0.24
116	143	Anaesthetized by amytal	327.7	329.9	2.2	0.67
128	119	Anaesthetized by amytal	516.2	518.8	2.6	0.50
78	160	Anaesthetized by amytal	104.2	104.3	0.1	0.10

killed by stunning, there is frequently a decidedly lower glycogen content in the muscle of the left leg which was last to be sampled, indicating that glycogenolysis occurs very rapidly in the dead organism.

Table 2 summarizes the results of different procedures in depleting the stores of muscle glycogen. The effects of fasting different lengths of time, of different types of work, of cold and of different periods of swimming are perfectly evident from the table. Thirty degrees Centigrade was considered to be about the optimum temperature of the water for swimming rats. Water at 25°C. produced fatigue and depletion of the glycogen stores much more quickly, but the animals reached the point of exhaustion so suddenly, or became so chilled and stiff, that they would abruptly quit swimming and drown.

Table 3 summarizes the average findings for liver and muscle glycogen content in the control rats: (a) normal, fed animals taken directly from the stock colony; (b) 24-hour fasted animals; (c) rats fasted for 24 hours and exercised

TABLE 2

Effect of fasting, cold and exercise on muscle glycogen

CONDITION OF RATS	NO. OF RATS	AV. WT. OF RATS	TYPE OF EXERCISE	AMOUNT OF EXERCISE	HOURS IN COLD	GLYCOGEN AS MG. OF GLUCOSE PER 100 GM. MUSCLE
		gm.				
Normal, fed	16	166	0	0	0	571.8
24-hour fasted	5	129	0	0	0	379.7
45-hour fasted	6	152	0	0	0	304.2
45-hour fasted	5	142	0	0	6	235.2
45-hour fasted	10	154	Running in revolving cage	1284 revolutions	6	300.6
81-hour fasted	4	105	Running in revolving cage	8917 revolutions	0	172.7
24-hour fasted	4	165	Running on treadmill	4 hours	0	308.5
24-hour fasted	3	185	Swimming	30 minutes	0	286.5
24-hour fasted	6	142	Swimming	1½ hours	0	193.2
24-hour fasted	7	128	Swimming	5 hours	0	93.3

TABLE 3

Glycogen content of control rats

CONDITION OF RATS	NUMBER OF RATS		AVERAGE WEIGHT OF RATS	MG. OF GLYCOGEN PER 100 GM. OF LIVER	MG. OF GLYCOGEN PER 100 GM. OF MUSCLE	CALCULATED TOTAL LIVER GLYCOGEN	CALCULATED TOTAL MUSCLE GLYCOGEN
	Liver	Muscle					
			gm.			*mg.*	*mg.*
(a) Normal, fed	4♀	13♀ 3♂	166	2778.6	571.8	138.4	474.6
(b) 24-hour fasted	3♀	5♀	132	33.8	379.7	1.3	244.9
(c) 24-hour fasted fatigued	19♀	19♀	144	21.5	70.9	0.9	51.0
(d) Fasted, fatigued, 1-hour recovery	19♀	19♀	147	25.4	101.0	1.1	74.2
(e) Fasted, fatigued, 2-hour recovery	16♀	16♀	144	23.5	119.8	1.0	86.3
(f) Fasted, fatigued, 3-hour recovery	15♀	15♀	154	69.4	121.6	3.2	93.6

by swimming until fatigued; (d), (e), and (f) rats fasted for 24 hours, fatigued by swimming, and allowed to recover for 1, 2 and 3 hours respectively. The values are expressed as milligrams per 100 gm. of tissue, and also as calculated total amounts, assuming (Cori, '31) that the muscles constitute about 50 and the liver only 3% of the body weight. Sixteen normal, fed rats showed a liver glycogen content of 2779.0 mg. per cent and a muscle glycogen of 572 mg. per cent. After 24 hours fasting the liver glycogen was reduced by one-third during this period of starvation. Fatiguing exercise decreased the liver glycogen only slightly, but brought the muscle glycogen down to a very low level, i.e., 71 mg. per cent, or about one-eighth of the amount in normal, fed animals. During the recovery period following fatigue, a small amount of glycogenesis occurs. The liver glycogen is not much affected until the third hour, when it reaches a level higher than that observed after fasting only. The muscle glycogen rises steadily during the first 2 hours, with little change in the third hour. These control values observed in the fatigued rats after 1, 2 and 3 hours recovery without food were taken as the base line in determining "new glycogen" formation, or the increase above the pre-formed level, in the sugar fed rats (see following paper).

DISCUSSION OF RESULTS

As a primary requisite in glycogen studies, the experimental animals should be of identical sex, of limited age range and previously maintained on a uniform diet. Stöhr ('32) noted a higher level of liver and muscle glycogen in male than in female rats after a 24–32-hour fast. The liver glycogen of the male rat diminished with increasing age, whereas the old females showed a marked increase in liver glycogen. Deuel, Gulick, Grunewald and Cutler ('34) confirmed the existence of significant sexual variations in carbohydrate metabolism. These authors found liver glycogen to be higher in fasting male than in female rats 24, 48 and 72 hours after glucose administration. There was no difference between the sexes

killed without fasting after a high carbohydrate diet. Muscle glycogen was not appreciably different in the two sexes.

The composition of the diet on which experimental animals have been previously maintained is a factor of considerable importance in establishing a base-line glycogen level (Greisheimer and Johnson, '30; Sahyun, Simmonds and Working, '34; MacKay and Bergman, '33). After having been weaned the rats used in this investigation were fed uniformly on the commercial chow and nothing else.

The time of fasting in the present work was limited to 24 hours, since this period certainly brings the animal into a postabsorptive condition and produces a sufficiently marked depletion of the glycogen stores. Longer fasting appears to be contraindicated, since the decrease in glycogen after 24 hours is very slow (22 mg. between the twenty-fourth and forty-eighth hour, according to Cori and Cori, '28) and since prolonged starvation diminishes the rate of absorption and utilization of carbohydrates (Cori, '27–'28; MacKay and Bergman, '33; Goldblatt, '25).

Since Page ('23) introduced amytal as an anaesthetic which is without effect on the blood sugar level of dogs or rabbits, it has been used with approval by many investigators. In the experience of this laboratory dogs in long experiments under amytal do show significant changes in blood sugar (Driver and Murlin, '41), but neither this type of effect, the interference with absorption reported by Olmsted and Giragossintz ('29), nor the inhibition of glycogen recovery noted by Long ('28) could come into consideration in the use of amytal as a means of securing tissue only.

In this investigation, the tissue samples were not frozen, contrary to the recommendation of numerous authors. It has been observed that freezing causes well-defined tetanic contractions of muscle, which Steiner ('35) found to result in a small loss of glycogen, reflected in a rise in both fermentable sugar and hexosemonophosphate.

After exhausting exercise the depleted glycogen reserves of liver and muscle are gradually replaced, even in the

absence of food. The processes involved are probably the same ones which are responsible for the increased storage observed during the nocturnal portion of the diurnal cycle, and may include, to some extent, migration of glycogen from the reservoirs in the skin and the bony and cartilaginous parts of the body.

Since Evans, Tsai and Young ('31) were unable to account for the combustion and liver glycogen deposition occurring in fasting cats recovering from experimental glycogenolysis, on the basis of observed changes in blood glucose, blood lactate and muscle glycogen, they concluded that the restoration must be at least partly due to processes of gluconeogenesis. Brand and Krogh ('35) also attributed the endogenous increase in liver and muscle, during recovery from exhausting work, to a small new formation of carbohydrate from non-carbohydrate sources. Although experimental proof was lacking, protein seemed a more probable source than fat.

The observation of Ågren et al. ('31) that the diurnal variation in liver glycogen is accompanied by a parallel variation in nitrogen output (i.e., increased deamination with increased glycogen deposition) indicates that glyconeogenesis may occur partly at the expense of body proteins during recovery from fatigue as well as during the normal diurnal cycle. The glycerol fraction of catabolized body fat possibly contributes its share. It is very likely also that lactic acid, accumulating in the tissues and blood as a result of the exercise, is a potential source of this "new glycogen." Its role, however, must be relatively unimportant in view of the work of Long and Grant ('30) showing that in the 24-hour fasted rat, where the carbohydrate stores are extremely low, the resynthesis of muscle glycogen after exercise is very slow, as compared with the rate at which lactic acid is removed from the blood.

No attempt was made in this investigation to prove that the recovery took place by glyconeogenesis either from fat or from protein. By analogy with previous work already cited, it is reasonably certain, however, that newly-formed carbohydrate must have contributed a share. The transfer of

glycogen from other tissues, such as the skin, should be controlled to make this conclusion final.

The values obtained in the tissue analyses of the control animals compare very favorably with the figures to be found in the literature. The liver glycogen content of 2.78%, found in the unfasted rats, is somewhat lower than the level of 3.53% reported by Deuel, Gulick, Grunewald and Cutler ('34), but can probably be explained on the basis of the carbohydrate level of the previous diet, and the time of day at which the samples were taken. The muscle glycogen content of 572 mg. per cent, found in the unfasted animals, is a normal figure. The liver glycogen value after 24 hours without food, as reported in the literature, varies from 0.06 to 0.70% for female rats. Long and Grant ('30) found a value of 0.08%. The slightly lower value found in this investigation, 0.03%, can probably be explained as above. The muscle glycogen content of 380 mg. per cent, found after a 24-hour fast, is almost identical with the figure of 383 mg. per cent reported by Deuel et al. ('34). Long and Grant's ('30) observation that "the changes in liver glycogen during and after exercise are so small that they are insignificant" is amply confirmed by the data of table 3. This work also bears out the finding of earlier investigators, who noted a marked decrease in muscle glycogen during exercise and a gradual reformation during recovery without food.

SUMMARY

The glycogen content of paired muscles removed from rats killed by a blow on the head was compared with that of animals anaesthetized with amytal. Much more closely agreeing results were obtained from the live anaesthetized animals.

The effect of fasting, of exposure to cold, and of various kinds and amounts of exercise (running ad libitum in revolving cages, forced running on a treadmill, and swimming in water at different temperatures) was investigated. A 24-hour fasting period reduced the liver glycogen to a very low

level, which was depressed only slightly more by the fatiguing exercise. It reduced the muscle glycogen by one-third; after 24 hours the rate of fall was much slower. Fatiguing exercise while fasting decreased the muscle glycogen content to a much lower level. During the recovery period after fatigue by swimming 8 hours, a small amount of glycogenesis occurred, notwithstanding the fasting state. The liver glycogen was not much affected until the third hour, when it reached a level twice as high as that observed after fasting only. The muscle glycogen rose notably during the first 2 hours, but only slightly in the third hour.

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GLYCOGEN FORMATION IN LIVER AND MUSCLE FROM GLUCOSE AND FRUCTOSE AFTER EXTREME MUSCULAR EXHAUSTION *

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THREE FIGURES

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The two principal monosaccharides occurring as such or in combination in human foods differ sufficiently in their chemical and physical properties to produce plainly measurable differences in their absorption rates, in their rates of combustion and their specific dynamic effects. How much under extreme need would they differ in restoration of glycogen to the tissues suffering most from muscular exhaustion? The previous study (Nutter, '41) showed that swimming was the best of several methods tried for producing an extreme reduction in the glycogen of muscles as well as of liver. Also that a slight amount of restoration of glycogen in both tissues occurs following extreme exhaustion without feeding. It turned out that this spontaneous recovery of glycogen without visible supply of carbohydrate was quite small and might have been neglected so far as the comparative effects of fed glucose and fructose were concerned. What could not be neglected, however, was the difference in rate of absorption of the two hexoses. An "index of glycogen" formation was therefore devised which takes into account both absorption rates and those of glycogenesis.

* This investigation was aided by a grant from the Corn Industries Research Foundation.

[1] The data in this paper were taken from a thesis submitted in partial fulfillment of requirements for the Doctorate of Philosophy, University of Rochester, June, 1938.

PROCEDURE

The experimental animals were fasted and fatigued by swimming exactly as the controls (Nutter, '41). After removal from the water, and drying, they were fed by stomach tube 2.5 cc. of the sugar solutions to be tested. All solutions were brought to 40°C. before feeding. In the earlier experiments, in which absorption was allowed to proceed for 2 hours, the fed sugars were 50% glucose [2] and 50% fructose.[3] The fructose feeding frequently resulted in diarrhea, and animals thus affected were discarded. In the next series, in which absorption proceeded for 3 hours, diarrhea invariably followed the feeding of 50% fructose. Consequently, the concentration of both sugars was reduced to 40%. Slight symptoms often appeared even then after fructose, and in such cases the excreta were collected and added to the unabsorbed residues from the gastrointestinal tract. At the end of the 1-, 2- or 3-hour absorption period the sugar-fed rats were anaesthetized and samples of muscle and liver were obtained. Especially after the 1-hour absorption period, the stomach was still so full that regurgitation often occurred during the operative manipulations. To prevent loss of solution through the mouth, the abdomen was opened as the first step, and the esophagus was closed off with an artery clamp, before the tissue samples were excised.

Absorption was determined in the usual way by the so-called [4] Cori ('25) method with all precautions taken to prevent possible losses.

The liver and muscle glycogen content was determined by the method previously described, final estimation of the sugar produced by acid hydrolysis of the glycogen and all other sugar determinations being made by the copper-iodometric

[2] Supplied by the Corn Industries Research Foundation.

[3] Pfanstiehl, C. P.

[4] This method of killing animals of similar weight at different intervals after feeding a uniform amount of a food and analyzing the contents of the alimentary tract in order to determine the progress of digestion and absorption was originally introduced by Schmid-Muhlheim (1879) and was used very successfully by Kugler ('19) for absorption of protein.

titration method of Shaffer and Somogyi ('33), with corrections for non-fermentable reducing substances where necessary.

EXPERIMENTAL RESULTS

As previously described, in the 2-hour experiments the sugars were fed in two different concentrations, first as 50% solutions, and later as equal volumes of 40% solutions. As shown in table 1, a decrease in the concentration of sugar fed, either glucose or fructose, is accompanied by a decrease in the total amount of sugar absorbed and also by a decrease in the amount of sugar absorbed per 100 gm. of body weight.

TABLE 1

Effect of concentration on sugar absorption (2-hour absorption period)

SUGAR	CONCENTRATION	AMOUNT FED	TOTAL AMOUNT SUGAR ABSORBED	SUGAR ABSORBED IN MILLIGRAMS 100 GM. BODY WEIGHT
	%	*mg.*	*mg.*	
Glucose	50	1014.6	580.2	442.7
Glucose	40	761.5	340.5	233.4
Fructose	50	1160.7	504.4	329.5
Fructose	40	791.8	287.7	202.2

It should be noted (table 2) that glucose is always absorbed at a more rapid rate than is fructose, and also that there is a falling off in the rate as absorption proceeds. When the absorption rate is plotted against time, including all experiments and all concentrations of the sugar solutions fed, the decrease in rate is observed in the third hour. When the absorption rate is plotted against time, including only those experiments in which approximately the same concentrations of sugar were fed, the decrease in rate occurs in the second hour, giving S-shaped curves (fig. 1).

Table 2 summarizes the findings for new glycogen formation in the liver, and presents the corresponding data for muscle, the values being expressed as milligrams of new glycogen per 100 gm. of tissue (actually calculated as milligrams of glucose). The results show that glucose exceeds

fructose somewhat in restoring muscle glycogen during the 3 hours of observation and also liver glycogen for the first 2 hours. During the third hour considerably more glycogen is deposited in the liver from fructose, than from the aldohexose. In view of the faster rate of absorption of glucose it was necessary, in order to compare the new glycogen forma-

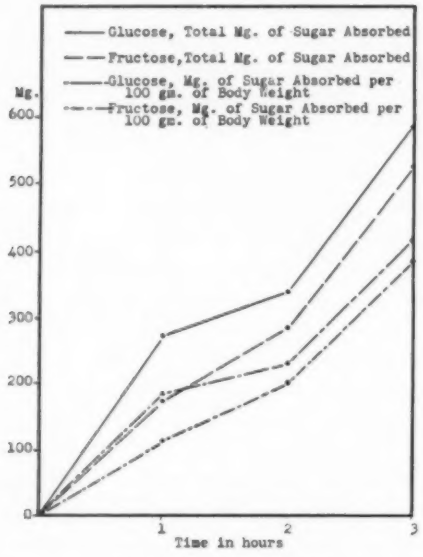

Fig. 1 Absorption of glucose and of fructose in 1-, 2- and 3-hour periods after feeding to exhausted rats.

tion from the hexoses on the basis of equal amounts of absorbed sugar, to establish an "index of glycogen formation," i.e., the ratio:

$$\frac{\text{new glycogen (expressed as milligrams glucose per 100 gm. of tissue)}}{\text{milligrams sugar absorbed per 100 gm. of body wt.}}$$

In the liver (table 2, fig. 2) the glycogen index for glucose is ten times that for fructose at the end of 1 hour. By the close of the second hour, fructose has nearly equalled glucose

TABLE 2

Glycogen formation in the recovery process after fatigue, "index of glycogen" formation in liver and leg muscles

CONDITION OF RATS	NO. OF RATS	AV. WT. OF RATS	MG. OF SUGAR FED	MG. OF SUGAR ABSORBED PER 100 GM. BODY WT.	MG. TOTAL GLYCOGEN PER 100 GM. TISSUE	MG. NEW GLYCOGEN PER 100 GM. TISSUE	INDEX OF GLYCOGEN FORMATION IN TISSUE	STANDARD DEVIATION, GLYCOGEN INDEX	STANDARD ERROR OF THE MEAN
		gm.							
				Liver					
Fasted, fatigued k. 1 hour after glucose	11♀	146	787.1	187.4	127.5	102.1	0.516	0.596	±0.180
Fasted, fatigued k. 2 hours after glucose	7♀	148	761.5	233.4	228.4	204.9	0.806	0.725	±0.274
Fasted, fatigued k. 3 hours after glucose	2♂ 12♀	142	825.8	415.2	804.5	735.1	1.689	1.216	±0.325
Fasted, fatigued k. 1 hour after fructose	13♀	154	792.0	115.1(61)	30.7	5.3	0.052	0.276	±0.077
Fasted, fatigued k. 2 hours after fructose	7♀	139	791.8	202.2(87)	185.3	161.8	0.750	0.880	±0.333
Fasted, fatigued k. 3 hours after fructose	1♂ 13♀	136	824.9	389.7(94)	1855.9	1786.5	4.605	1.678	±0.448
				Leg muscle					
Fasted, fatigued k. 1 hour after glucose	11♀	146	787.1	187.4	165.0	64.0	0.327	0.379	±0.114
Fasted, fatigued k. 2 hours after glucose	3♂ 13♀	139	903.9	351.1	377.3	257.5	0.661	0.394	±0.099
Fasted, fatigued k. 3 hours after glucose	2♂ 13♀	142	825.8	415.2	415.6	294.0	0.687	0.307	±0.079
Fasted, fatigued k. 1 hour after fructose	13♀	154	792.0	115.1(61)	118.3	17.3	0.111	0.637	±0.177
Fasted, fatigued k. 2 hours after fructose	2♂ 15♀	147	1008.8	277.1(79)	255.2	135.4	0.459	0.340	±0.082
Fasted, fatigued k. 3 hours after fructose	1♂ 13♀	136	824.9	389.7(94)	367.8	246.2	0.644	0.237	±0.063

k. = killed.

Figures in ( ) = percentage rates as compared with glucose.

in glycogenic power in the liver, and at the end of the third hour, fructose shows a glycogen index over two and one-half times that for glucose. In the muscle the differences are not so remarkable. Although the glycogen index for glucose is almost three times that for fructose at the end of the first

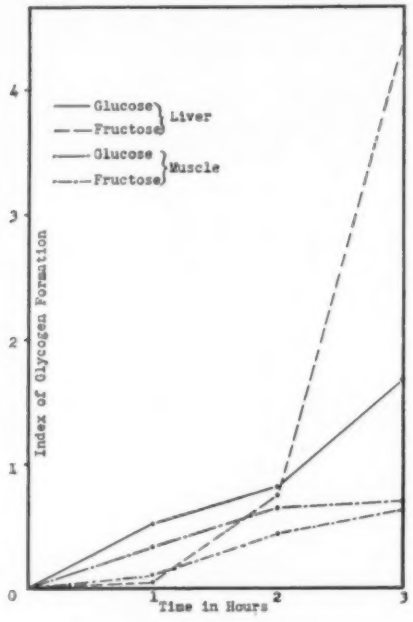

Fig. 2 Glycogen formation at intervals after feeding glucose and fructose, immediately following exhaustive fatigue, expressed as "glycogen index" (see text).

hour, after 2 hours it is less than one and one-half times as great, and by the end of the third hour, fructose shows almost as great a glycogenic capacity as glucose.

Assuming that the muscle tissue accounts for 50, and the liver for 3% of the body weight (Cori, '31), the total amount of new glycogen formed in liver and muscle from glucose and fructose was estimated, and the percentage of the ab-

sorbed sugar which was deposited as liver and as muscle glycogen was calculated. In total amount of new glycogen formation (these results are omitted in the interest of brevity) fructose falls somewhat behind glucose during the first 2 hours in the liver, but forges far ahead in the third hour. In the muscle, fructose lags behind during each of the 3 hours.

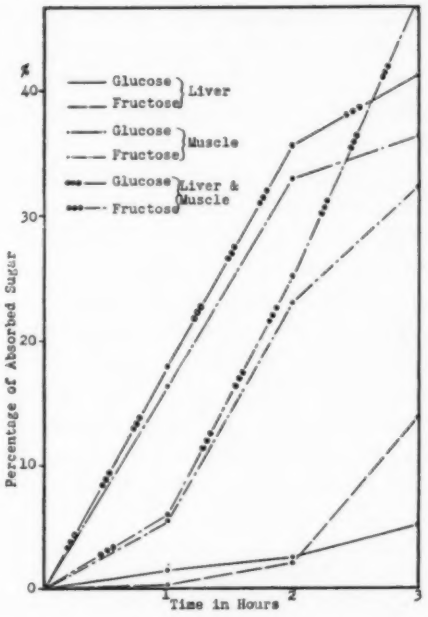

Fig. 3 Glycogen formation at intervals after feeding glucose and fructose, immediately after exhaustive fatigue, expressed as percentage of absorbed sugar laid down in liver and muscle and in both together.

When the percentage of absorbed sugar that is laid down as liver and muscle glycogen is calculated, glucose is found to be nearly ten times as effective as fructose in forming liver glycogen during the first hour (fig. 3). In the second hour fructose is almost as active as glucose, and by the end of the third hour the percentage of absorbed fructose deposited

in the liver is over two and one-half times the value for glucose. In the muscle the activity of the absorbed fructose in building up glycogen is less than half that of glucose in the first hour. In the second hour, fructose still lags behind, while in the third hour the keto sugar closely approaches glucose. Plotting the total percentage of absorbed sugar deposited in liver and muscle against the time in hours, one observes (fig. 3) that the rate is constant for glucose during the first 2 hours, falling off in the third hour. In the case of fructose, there is a lag in the first hour, followed during the last 2 hours by a constant rate which, curiously, very nearly parallels the initial rate for glucose.

Comparison of the glycogenic powers of the two hexoses on the basis of equal amounts of sugar absorbed, by means of the index of glycogen formation, or comparison of the percentage utilization of the absorbed sugars in total formation of liver and muscle glycogen, indicates that glucose is superior to fructose as a glycogenic agent in the early stages of recovery from fatigue, even when allowance is made for the difference in rates of absorption. However, as the time of recovery is prolonged, i.e., to 3 hours, the difference between the glycogenic capacities of the two sugars decreases in muscle, and in the liver fructose is stored to a much greater extent than is glucose.

DISCUSSION OF RESULTS

Cori's ('25) conclusion that the rate of absorption of the hexoses is independent of the absolute amount and concentration of sugar present in the intestine, giving a straight-line relationship between absorption rate and time, is definitely not confirmed by the observations on fatigued rats herein reported (table 1). Cori also concluded from data based on hourly determinations of the absorption coefficient (absorption per 100 gm. of body weight per hour), that fructose is absorbed at only 0.43 the rate observed for glucose. Burget, Moore and Lloyd's ('32) observation that glucose is absorbed only 9% more rapidly than fructose was made on a much

larger and better controlled series of animals. In the present investigation using fatigued rats, in which the absorption coefficients varied widely, the average results showed fructose to be absorbed at 61% of the rate for glucose during the first hour, 79 to 87% during 2 hours, and 94% during 3 hours (table 2). Factors responsible for the rather wide deviations observed in the amount of sugar absorbed during any given time interval may include: irregularities in the emptying rate of the stomach, variations in the concentration and pressure of the solution in contact with the intestinal mucosa due to secretion of variable amounts of water into the intestinal lumen, and varying degrees of disturbance of gastrointestinal motility due to the psychic effects of the exercise.

The data also contradict the conclusion of Cori ('25) and Cori, Cori and Goltz ('29) that the absorption rate of the hexoses is constant. The falling off in rate as absorption proceeds, as observed in the present investigation, cannot be due to lack of sufficient sugar to maintain the initial rate, for at the end of the third hour the gastrointestinal tract still contained more sugar than had been absorbed during the first hour. The significance of Cori's conclusions diminishes considerably when one considers that they are based on observations made on groups of rats each containing an average of less than five animals, and that his recorded data show a range of variation between the maximum and minimum "absorption coefficients" which in some groups reaches or exceeds 50% of the average value. Obviously the average results from such small groups, in which so wide a range of variation occurs, cannot be considered very significant. Similar investigations in our own and in other laboratories show these wide animal variations, indicating that long series of experiments are necessary before any valid conclusions can be drawn from the average results. The S-shape of the absorption rate curve obtained by plotting only those experiments in which approximately equal amounts of sugar were fed corresponds well with the results reported by Feyder and Pierce ('35) for average total absorption of glucose and sucrose by unfatigued

rats during 1-, 2- and 3-hour periods. Any explanation of the S-shape of the curve must of necessity be rather hypothetical. However, the following mechanisms might be suggested. In the initial stages absorption proceeds very rapidly, due to the high concentration of the sugar solution fed. This concentrated solution is extremely irritating to the gastrointestinal mucosa, and stimulates the secretion (aided by osmotic pressure) of large quantities of water into the intestinal lumen.[5] Due to the diluting effect of the alimentary secretions, absorption of sugar proceeds more slowly during the second hour. But the water is reabsorbed at a rate faster than the sugar passes through the intestinal wall, so that by the third hour the solution is again fairly concentrated, and absorption of sugar proceeds at a faster rate. Other factors besides concentration are probably involved, including pressure effects and the emptying rate of the stomach.

In order to determine the significance of the differences in the effectiveness of glucose and fructose in forming new liver and muscle glycogen, the data were subjected to statistical analysis (Davenport and Ekas, '36). The actual difference between the means was compared with the square root of the sum of the squares of the two standard errors of the means, expressed by the formula:

$$\sqrt{SE_{M_1}{}^2 + SE_{M_2}{}^2}$$

A significant difference between two means is one which exceeds twice this root, i.e.,

$$\frac{M_1 - M_2}{\sqrt{SE_{M_1}{}^2 + SE_{M_2}{}^2}} > 2$$

This ratio will be referred to as the significance ratio.

[5] That this process does occur is a proved fact. One hour after feeding these strong solutions, the stomach and upper intestine are found to be greatly distended. Within the following hour much of the fluid is reabsorbed. Abbott, Karr and Miller ('36) found glucose concentrations in the jejunum and ileum of human subjects ranging from 0 to 5%, regardless of the volume and concentration of solution ingested. The data showed that a very rapid inflow of fluid occurred from the walls of the stomach and duodenum.

Calculations thus performed (table 3) show that in the liver the superiority of glucose over fructose in restoring glycogen, during the first hour of recovery in the fatigued rat, is statistically significant, the actual difference being considerable, and the significance ratio being 2.4. By the close of the second hour fructose has nearly equalled glucose in glycogenic power in the liver, since the actual difference in the means (mean index of glycogen formation and mean percentage utilization) is very small and the significance ratio of the difference in the means is only 0.13. The superiority of fructose at the end of the third hour is very significant, with a ratio of 5.3. Although the average results for new glycogen formation in muscle indicate a greater effectiveness for glucose, especially in the first hour, the difference between the two sugars is not significant at any time. That the value of the significance ratio falls below 2.0 for the two sugars in building up muscle glycogen is partly to be accounted for, at least in the first 2 hours, by the extremely wide variations observed in the individual experiments. It is very likely that extension of the series to include a much larger number of animals might prove glucose to be significantly superior to fructose as a muscle glycogen former in the early stages of recovery after fatigue. The experimental groups reported here are relatively large, each containing eleven to nineteen animals, with the exception of 2-hour liver determinations on seven animals for each sugar.

Several causative factors may be suggested in explaining the initial slow rate of glycogen production. Perhaps compounds more fundamental than glycogen to the organism must first be replenished. Depression of insulin production during the previous fasting period is probably involved. In the early stages of the recovery process a high rate of carbohydrate combustion is quite conceivable. As a result of the prolonged period of strenuous exercise, there probably exists a decided condition of acidosis, due to accumulation of lactic acid and acetone bodies. Thus it seems reasonable to expect that a considerable carbohydrate combustion would be necessary

TABLE 3

Statistical comparison of glycogen formation from glucose and fructose

	NO. OF EXPTS.	MEAN	STANDARD DEVIATION	STANDARD ERROR OF THE MEAN	$\sqrt{S.E._{M_1}^2 + S.E._{M_2}^2}$	$M_1 - M_2$	SIGNIFICANCE RATIO $\frac{M_1 - M_2}{\sqrt{S.E._{M_1}^2 + S.E._{M_2}^2}}$
Index of glycogen formation in liver							
1-hour glucose	11	0.516	0.596	±0.180	0.196	0.464	2.367
1-hour fructose	13	0.052	0.276	±0.077			
2-hour glucose	7	0.806	0.725	±0.274			
2-hour fructose	7	0.750	0.880	±0.333	0.431	0.056	0.130
3-hour fructose	14	4.605	1.678	±0.448			
3-hour glucose	14	1.689	1.216	±0.325	0.553	2.916	5.273
Per cent of absorbed sugar deposited as liver glycogen							
1-hour glucose	11	1.54	1.80	±0.54			
1-hour fructose	13	0.16	0.81	±0.22	0.58	1.38	2.379
2-hour glucose	7	2.42	2.18	±0.82			
2-hour fructose	7	2.25	2.63	±0.99	1.29	0.17	0.132
3-hour fructose	14	13.82	4.95	±1.32			
3-hour glucose	14	5.07	3.65	±0.98	1.64	8.75	5.335
Index of glycogen formation in muscle							
1-hour glucose	11	0.327	0.379	±0.114			
1-hour fructose	13	0.111	0.637	±0.177	0.211	0.216	1.024
2-hour glucose	16	0.661	0.394	±0.099			
2-hour fructose	17	0.459	0.340	±0.082	0.129	0.202	1.566
3-hour glucose	15	0.687	0.307	±0.079			
3-hour fructose	14	0.644	0.237	±0.063	0.101	0.043	0.426
Per cent of absorbed sugar deposited as muscle glycogen							
1-hour glucose	11	16.36	18.96	±5.72			
1-hour fructose	13	5.58	31.87	±8.84	10.35	10.78	1.042
2-hour glucose	16	33.06	19.66	±4.92			
2-hour fructose	17	22.95	16.98	±4.12	6.41	10.11	1.577
3-hour glucose	15	36.12	12.95	±3.34			
3-hour fructose	14	32.20	11.85	±3.17	4.60	3.92	0.852

in order to accomplish the disposal of the products of muscle metabolism. Moreover, probably some period of time is required for the liver to return to its optimal conditions for glycogen formation.

The greater lag shown by fructose may indicate that certain time-consuming rearrangements of the molecule must occur before it can be polymerized to the polysaccharide. Other factors may be involved, such as the lower renal threshold for fructose, a more rapid conversion of fructose to fat, and a greater ease of oxidation of fructose. The conclusion, arrived at in earlier investigations, that fructose is superior to glucose as a glycogenic agent, has generally been based on analyses of the liver made several hours after administration of the sugars, without regard for the immediate effects or the concurrent changes in the muscle.

Why fructose should produce a higher maximum of liver glycogen than glucose, of which there is more available, can hardly be decided on the basis of our present knowledge. It might be postulated that the polymerization occurs first in the liver, and that the glycogen deposited from fructose is less readily hydrolyzed than that from glucose, for transfer to other parts of the body. However, there is at present no evidence of chemical or biological differences in the glycogens formed from the two sources.

SUMMARY AND CONCLUSIONS

Rats, fasted and fatigued by swimming were fed glucose or fructose solutions by stomach tube. After 1-, 2- and 3-hour absorption periods the animals were anaesthetized with amytal, liver and muscle samples obtained, and the unabsorbed residues in the gastrointestinal tract were collected. The amount of sugar absorbed, and the increases in liver and muscle glycogen above the pre-formed control level were determined. Fructose was found to be absorbed at a somewhat slower rate than glucose by the fatigued rat, but both sugars showed a falling off in absorption rate as the time was prolonged. To allow for the inequalities in rate of absorption,

the new glycogen formation in liver and muscle from glucose and fructose was compared on the basis of the "index of glycogen formation," which takes into account both absorption rates and glycogenesis rates, as well as on the basis of percentage of the total amount of sugar absorbed that is deposited in the total liver and muscle glycogen stores. Glucose was found to be superior to fructose in rebuilding the liver glycogen stores during the early stages, i.e., the first hour, of recovery after exhausting fatigue, the difference being statistically significant. Fructose approaches glucose in activity in the liver in the second hour, and in the third hour is much more effective than glucose. In muscle the average results for new glycogen formation indicate a greater effectiveness for glucose, especially in the first 2 hours, but the difference between the two sugars is not statistically significant at any time.

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STUDIES ON THE VITAMIN C METABOLISM OF FOUR PRESCHOOL CHILDREN [1]

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There have been very few reports of quantitative studies on the vitamin C requirements of healthy preschool children. Everson and Daniels ('36) reported a study of three preschool boys, for twelve 5-day periods on levels of 50 to 214 mg. intake. They considered that 6 to 7.5 mg. per kilogram were required to give optimal retentions in these children. Widenbauer ('37) found that the "Zusätzlichen Tagesverbrauches" value for a normal 2½-year-old boy averaged 21 to 22 mg. per day for seven weekly periods. Other studies on the vitamin C requirements of preschool children have been short-time investigations on larger numbers of convalescent children in hospitals rather than detailed studies on a few healthy ones, and differences in the methods employed make comparison difficult. Several of these studies will be discussed later in this report.

Because of wide differences in the requirement reported by the various workers, it seemed important to carry out further studies on the needs of the normal preschool child.

EXPERIMENTAL

Subjects. Four healthy preschool children, two boys and two girls, were selected for this study. Physical examinations given at the beginning and at the end of the experiment showed them all to be normal according to present standards

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of measurement. Data on their sex, age, weight, height, placement on Grandprey's ('33) charts and basal metabolic rate at the end of the experiment are given in table 1. This period was selected to be reported since the basal tests were made at this time.

TABLE 1

Physical status of subjects

SUBJECT	SEX	AGE	WEIGHT	HEIGHT	GRANDPREY'S PLACEMENT FOR			BASAL METABOLIC RATE		
					Wt. for age	Ht. for age	Wt. for ht.	Calories	Deviation from standard for	
									Weight [1]	Height [1]
		mo.	kg.	cm.	%	%	%		%	%
D.T.	M.	64	17.7	106.7	50	45	70	791	—1	—3
J. S.	F.	59	20.6	109.7	over 90	85	over 90	763	—11	—5
C. M.	F.	43.7	17.8	105.4	over 90	over 90	85	830	+4	+7
D. S.	M.	43.2	16.8	99.1	85	70	90	856	+11	+10

[1] See Talbot's Standards for children ('38).

Caloric intake. During the first weeks of the study an attempt was made to give the two older children, D. T. and J. S., 25% more food than the younger ones. However, it was difficult to get them to take it, and consequently the amount of food given was cut to the lower level for all four children. The average daily caloric values for the food actually eaten were calculated from Waller's tables ('35). The following values represent the daily averages for the last 3 months of the experiment, the period covered in detail by this report: D. T., 1218 Calories; J. S., 1217 Calories; C. M., 1235 Calories, and D. S., 1230 Calories. On the basis of the basal metabolic tests it is not surprising that the older children did not want more food, since their basal requirement was lower in both cases, and their activity was about the same as that of the other two children.

Diet. The basal diet met the recommended optimal standards in all known nutrients except vitamin C. The foods used and their vitamin C content are given in table 2. The food other than milk contained 3 to 6 mg. ascorbic acid per day, and an additional 10 to 14 mg. per day, depending on the sample,

were supplied by the 800 cc. of specially prepared milk used in the study [2]. This milk which was furnished by the Dairy Department of Cornell University, had the oxygen removed by a method developed by Professors Sharp, Hand and Guthrie ('39), a process which preserves the ascorbic acid content and prevents the development of any oxidized flavor. The milk was prepared weekly by Dr. Guthrie and samples were tested by him for ascorbic acid and flavor at the beginning and end of the week to check its keeping quality. Canned fruits, vegetables, salmon and chicken were used, and all foods were bought in quantity whenever practicable. Several analyses were made of each food included in the diet to check the vitamin C content and little variation was found between the analyses. Average values were used in table 2. All menus were repeated weekly. Crystalline ascorbic acid [3] was added to the basal diet at levels from 200 mg. per day down to 15 mg. per day. The ascorbic acid was given with the milk and the additions were regulated so that not more than 50 mg. were added at any one time, e.g., the test dose of 200 mg. was given in 50 mg. portions in the milk at mealtime, and following the afternoon nap.

Urine collections. Each urine specimen was separated into two equal portions. One, which was used for the vitamin C determinations, was placed in a brown bottle and acidified immediately with 10% by volume of 2 N sulfuric acid containing 2% metaphosphoric acid. The other was preserved with thymol for pH determinations and mineral analyses. All samples were placed in the refrigerator as soon as preserved.

Vitamin C determinations. Daily determinations of the vitamin C output were made on 24-hour samples of the acidified urine. The following modifications were made in Bessey's method ('38) with the Evelyn photoelectric colorimeter. To

[2] The vitamin C values of the milk reported here are 3 mg. per liter lower than those reported by Sharp et al. for the same milk due to the difference between values obtained by titration and by the Evelyn photoelectric colorimeter.

[3] Hoffman LaRoche.

TABLE 2

Basal foods and their vitamin C values

FOOD	AMOUNT	VITAMIN C		DAYS PER WEEK FOODS WERE USED
	gm.[1]	*mg./100 gm.*	*mg./sample*	
Cereals				
Bread	36	...	...	7
Triscuits	16	...	...	7
Ralston	60	...	...	2
Oatmeal	60	...	...	3
Shredded Ralston	20	...	...	2
Rice	32	...	...	6
Macaroni	32	...	...	1
Meats				
Beef	32	0.92	0.29	4
Salmon	32	0.0	0.0	1
Chicken	32	0.71	0.23	1
Veal heart	32	1.58	0.51	1
Vegetables				
Beets	32	3.08	0.99	3
Peas	32	6.25	2.00	3
Carrots	32	0.97	0.31	4
Beans (Wax)	24	3.07	0.74	4
Potato	32	3.16	1.01	7
Fruits				
Prunes	52	0.53	0.28	3
Prune juice	8	1.43	1.03	3
Applesauce	60	1.79	1.07	4
Peaches	52	1.58	0.82	3
Peach juice	8	2.00	0.16	3
Pears	52	1.06	0.55	4
Pear juice	8	1.44	0.12	4
Egg	32	...	...	7
Milk	800 cc.	13–17/liter[2]	10–14	7
Cod liver oil	1 tsp.	...	...	7

Curd free butter[3], sugar, plum and quince jelly were given ad libitum

[1] All weights are for cooked food.

[2] See text.

[3] Prepared for us by Dr. E. S. Guthrie, Professor of Dairy Industry, Cornell University.

samples of urine preserved as indicated above, equal volumes of 2% metaphosphoric acid instead of 6% metaphosphoric acid were added. The H_2SO_4 used to preserve the urine did not interfere with the vitamin C determinations on addition of the 2% metaphosphoric acid. Duplicate solutions were prepared for each sample and triplicate readings made on each. The average of these six readings was used for the "Gs" and "Gsr" values. The "Gb" values were determined for the respective "Gsr" averages at the end of all analyses, using one tube of dye and blank. The value for K_1 as determined for our colorimeter was found to be 0.088 ± 0.002.

For the food analyses, Bessey's method ('38) was followed.

Although Chinn and Farmer ('39) showed that the feces of adults contain about 5 mg. of ascorbic acid daily, they found that in the normal individual large variations in the dietary intake affected the fecal excretion only slightly. Consequently no attempt was made to measure this excretion.

Plan of the experiment. Through the employment of graduate students trained in child care and the cooperation of the Family Life Department of the College, every effort was made to give the children happy normal experiences throughout their stay in the laboratory.

A preliminary period of about 10 days was used in which the children became adjusted to their new surroundings and new companions. During this period they learned to eat the foods included in the diet, to clean their plates, etc.; and they were given 50 mg. per day of crystalline ascorbic acid in addition to that in the food to insure tissue saturation.[4] The early periods on the 66 mg. level noted in table 3 were omitted in table 4 since they were of only 1-week duration and showed results almost identical with those obtained on the 2-week periods.

[4] The term "saturation" as used throughout this report refers to the condition in which the tissues have taken up from the blood stream all the vitamin C which they are capable of storing, so that on a constant high intake of the vitamin a relatively large and constant amount is eliminated in the urine.

Throughout the study each period was concluded by a test dose of 200 mg. to check tissue saturation. The periods were of either 1 or 2 weeks duration as indicated in table 4. In computing averages the first 2 days following a test dose were omitted to eliminate the excessive excretion resulting from the larger intake.

These results represent a total of thirty-two of the periods studied, eight for each child.

TABLE 3

Twenty-four hour excretion of ascorbic acid in response to 200 mg. test dose following periods on various levels of ascorbic acid intake

DAILY INTAKE OF ASCORBIC ACID	D.T.	J.S.	C.M.	D.S.	AVERAGE
mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
115	162	141	150	123	144
92	133	142	138	144	139
66	130,129,118	119,114,133	127,141,146	132,112,132	128
50	130	114	140	129	128
42	112+	138	132	143	131
31	107,109	73,112	113,119	89,119	105
17	83	72	....	50	68

RESULTS

The urinary responses to 200 mg. test doses following 1 or 2-week periods on various levels of ascorbic acid intake are shown in table 3. If an excretion of 50% of the intake is considered as the criterion of saturation, it is evident that on an intake of 115 mg. down to 42 mg. per day the tissues were saturated in all the children, but that on the 31 mg. level it was only following the second period at this level that D. S. and J. S. were saturated. At the 17 mg. level the tissues were not saturated in any of the children. It might be said, then, on the basis of their responses to test doses, that a daily intake of 31 mg. appears to be the marginal level for saturation in these children.

Table 4 summarizes the results for the average daily urinary responses of the four children on the various levels of ascorbic acid intake. During any week represented by these data,

except the week on the 115 mg. level, the variation in ascorbic acid excretion for any of the children averaged 6 mg. with a standard deviation of ± 3 mg. Variations at the highest level (115 mg.) were from 13 to 37 mg.

TABLE 4

Average daily excretion and "utilization" of ascorbic acid on various levels of intake

PERIOD	1	2	3		4		5		6		7		8
INTAKE IN MG.	115	92	66	66	50	50	41	43	32	31	31	32	17
Urinary excretion in mg.													
D. T.	77	59	38	32	26	20	17	19	9	11	14	11	6
J. S.	82	46	37	33	25	24	15	16	9	7	8	10	5
C. M.	90	61	39	39	29	27	18	20	11	7	10	10	6
D. S.	74	66	35	33	26	25	18	16	10	8	9	10	5
av.	81	58	37	34	26	24	17	18	10	8	10	10	5
"Utilization" in mg.													
D. T.	39	33	28	34	25	30	24	24	23	20	17	21	11
J. S.	34	46	29	33	26	26	26	27	23	24	23	22	12
C. M.	26	31	27	27	22	23	23	23	21	24	21	22	11
D. S.	42	26	31	33	25	25	24	27	22	23	22	22	12
av.	35	34	29	32	24	26	24	25	22	23	21	22	11
"Utilization" in %													
D. T.	34	36	42	51	49	60	58	56	72	64	55	66	65
J. S.	29	50	44	50	51	52	63	63	72	77	74	69	71
C. M.	22	34	41	41	43	46	56	53	66	77	68	69	65
D. S.	36	28	47	50	49	50	56	63	69	74	71	69	71
av.	30	37	43	46	46	52	58	59	70	73	67	68	68

Table 4 also includes the values for the "utilization" of the ascorbic acid. This term "utilization" is used arbitrarily to refer to the difference between intake and excretion. The mean value for the "utilization" level for the four children on intakes of 31 to 50 mg. is 23 mg. with a standard deviation of ± 2 mg. Thus the "utilization" on the 31 mg. level is about 74% of the intake, but on the 50 mg. level it is only about 47% of the intake. In no case was the average efficiency during any given week greater than

77%. On the basal diet alone the "utilization" did not exceed this figure although the tissues were not saturated. This may be considered another evidence that a daily intake of about 30 mg. is the marginal level required for saturation, since at this level the children were able to maintain tissue saturation with the maximum efficiency in the utilization of the ingested ascorbic acid.

DISCUSSION

Workers who have studied the vitamin C metabolism of children differ markedly in their criteria for tissue saturation. Two groups, Keith and Hickmans ('38) and Parsons ('38), used 500 mg. as the test dose, and considered the excretion of 50 mg. in the next 24 hours as the criterion of saturation. This seems a very low percentage in the light of data reported here for much lower intakes of the vitamin. Both groups determined the vitamin C by titration with 2–6 dichlorophenolindophenol.

Widenbauer ('37) considered that on an intake of 200–500 mg. determined by body weight and expected deficiency, saturation was reached when at least 50% of the half-day dose was excreted in 12 hours. He also used the titration technic, but his test dose and percentage values correspond more closely to those in this report.

Probably the most thorough study on the effect of the differences in the size of the test dose and the criteria of saturation is found in Baumann and Rappolt's report ('37). They showed that using 25 mg. per kilogram (or about 500 mg. for children of preschool age) no vitamin C deficit existed if 29–59% of the intake was excreted within 24 hours. In one subject on a 200 mg. intake 43% excretion in 24 hours was considered indicative of saturation whereas on a 100 mg. test dose the same subject excreted 90% in the same length of time. They found much greater variation on the higher test doses. It should be mentioned that the vitamin C content of the diet was not considered in their figures, and that the titration method was used.

No quantitative studies on the requirements for tissue saturation in children have been found in which the photoelectric colorimeter was used for the determination of vitamin C. Therefore it was necessary to set up our own criterion for saturation. Belser, Hauck and Storvick ('39), who studied saturation in adult subjects using the titration technic, allowed each individual to set his own standard for comparison. However, it will be noted that at least 50% of the test dose of 400 mg. was excreted when their subjects were saturated. By referring to table 3 it can be seen that if the individual response to the test dose following a period on 66 mg. is taken as the standard for comparison in these four children, all but D. T. equal or pass this standard down to the 31 mg. level. The value 112 for D. T. may be lower than it should be, due to a slight loss in his morning specimen in the laboratory before analysis. On this basis, one test on D. S. and J. S. would still show saturation on the 31 mg. level, although D. T. and C. M. would have values slightly below saturation at this level. Further tests will be made to establish if 50% excretion is a fair test for saturation, but since the above comparison with the method of Belser, Hauck and Storvick ('39) shows similar results, the conclusion that a daily intake of about 30 mg. is the marginal level for tissue saturation seems valid on either basis.

The term "utilization" is used arbitrarily to cover that portion of the ascorbic acid ingested which is not excreted in the urine. It is conceded that a part of this is probably excreted in the feces (Chinn and Farmer, '39), part may be decomposed in the gastrointestinal tract or within the body after it is absorbed, and part may replace some of the vitamin stored in the body, but that amount seems necessary for bodily function.

In this experiment little change was found in the amount of ascorbic acid "utilized" on intakes ranging from 30 to 50 mg. The question arises whether the increased "utilization" noted on higher levels is real or apparent, i.e., there might be a greater destruction of the vitamin at these higher levels. Better tests for measuring function and partition of

vitamin C within the body will be necessary before this question can be answered.

Comparison of the figures in the present study with those of Everson and Daniels ('36) at the 50 mg. level shows interesting differences. The present "utilization" figures were 22–30 mg., those for Everson and Daniels 36–40 mg. These differences may easily be explained on the basis of previous saturation of the tissues in this experiment. The importance of this factor had not been recognized when the earlier work was done. It was only when the intake levels reached 200+ mg. that the excretion values were in the range found in the present study. This might suggest that in the earlier study it was only when this level of intake was reached that the tissues were saturated. Since the tests on the 200+ mg. levels were the last ones made there was no check on excretion values following saturation.

It is of interest to note also that the greatest average efficiency of retention for any 5-day period in Everson and Daniels' tests was 77% for G. E. on a 50 mg. level on 12/15/36, the same value found by us with J. S. and C. M. on the 31 mg. level. Further observations on more children are needed before conclusions can be drawn but it can be stated that in these studies on seven preschool children over a 5–6 month period the efficiency of "utilization" or retention of ascorbic acid was not greater than 80% of the vitamin ingested regardless of the intake level. In four of the children the tissues were saturated except on the lowest level fed, but in the other three they were probably somewhat below saturation.

In contrast to this work with children the studies of Kellie and Zilva ('39) and Ralli, Friedman and Sherry ('39) with adults may be cited. Kellie and Zilva found that if the tissues had been saturated previously the retention on a 50 mg. daily intake was 70%, but if a state of "unsaturation" existed as much as 90% was retained even after 17 days at this level. Ralli et al. found retention values averaging 87 to 94% over a period of 140 days for one subject, and of 86 to 92% over a period of 127 days for a second one. This might indicate that in adults the ability to conserve (or destroy) ascorbic acid is greater than that found in children.

Although Widenbauer ('37) does not give the details of his experiment, our figures for vitamin C requirement are in essential agreement with his. He concluded that 22 mg. ascorbic acid were needed daily by a normal 2–3-year-old boy in addition to the experimental diet, low in vegetables and free from fruits. Our figure of 31 mg. per day would allow 9 mg. in his diet which is a plausible figure.

The requirement for vitamin C in these four children does not seem to be related to sex, as D. T. and D. S. were boys, J. S. and C. M. girls; nor is it affected by age at least within the range of 15 to 20 months covered by our subjects. It does not seem to be related to body weight, as their weights varied 3.5 to 4 kg. throughout the experiment. C. M. gained weight much more rapidly than the other children, increasing 2.5 kg. as compared with 0.5 to 1 kg. each for the other three children, but she showed no higher retention of vitamin C than the rest at any period in the study. Ascorbic acid requirements showed no correlation with the basal metabolic rates. The two children showing unsaturation after the first period on 31 mg. differed in age by 15 months, in weight by 4 kg. and in B.M.R. from +11% for the younger boy to —11% for his older sister. Ralli et al. ('39) also concluded that weight did not affect the vitamin C requirement as A. T. gained 20 pounds during the 200 days on the experiment with no effect on the vitamin C needs.

SUMMARY AND CONCLUSIONS

Four preschool children were studied on various levels of ascorbic acid intake in an otherwise constant diet. After it was established that their tissues were saturated with vitamin C by checking their responses to test doses of 200 mg. of pure ascorbic acid, the level of intake was varied as follows: 115, 92, and 17 mg. were used for 1-week periods, and 66, 50, 43, and 31 mg. for 2-week periods. The 31 mg. level was repeated. Each period on a new level was followed by a test dose to recheck tissue saturation. The results of the study may be summarized as indicated below.

1. A daily intake of 31 mg. of ascorbic acid was the marginal level to insure tissue saturation in these four children.

2. The "utilization" of ascorbic acid was relatively constant at levels of intake from 31 to 50 mg. and the average value for these four children for four 2-week periods was 23 ± 2 mg.

3. The per cent retention or "utilization" found in these children was lower than those reported in the literature for adults.

4. The requirement was not related to sex, age or body weight, at least within the limits found in these children.

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THE BIOLOGIC RESPONSE OF CHICKENS TO CERTAIN ORGANIC ACIDS AND SALTS WITH PARTICULAR REFERENCE TO THEIR EFFECT ON OSSIFICATION

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Interesting developments are now appearing in the literature as a result of continued studies on the relation of acid and alkaline-ash foods to the etiology of rickets and the metabolism of vitamin D.

The earlier work on this problem has been presented and summarized by Shohl et al. ('28, '31, '32). Among other things it has been reported that the cation-anion balance of the blood is normal in clinical rickets, infantile tetany and, except in special cases, in experimental rickets. Later work indicated that certain cationogens and anionogens have, however, a marked effect on such pathologic states. Hamilton and Schwartz ('33) reported that rickets was completely prevented in rats on a rickets-producing diet by the addition of the cationogen, acid sodium tartrate. In rats on a non-rachitogenic diet rickets was produced by a mixture of the anionogens, ammonium chloride and ammonium carbonate. Hamilton and Dewar ('37) extended this investigation and demonstrated that sodium citrate, sodium bitartrate, citric acid, and tartaric acid all prevented the development of rickets in rats maintained on a rachitogenic diet.

The principal conclusion from the above mentioned researches, that some organic acids and the salts of these acids when fed to rats on a rachitogenic ration will permit normal ossification even in the absence of any form of vitamin D,

has been verified by Hathaway and Meyer ('39), Day ('40) and Shohl ('37). This latter author fed eight basal diets, each one having a different calcium to phosphorus ratio, and found that ammonium chloride-ammonium carbonate mixtures produced rickets with rations which previously were non-rachitogenic or intensified the rickets produced by the rachitogenic feeds. Citric acid-sodium citrate mixtures added to rachitogenic diets protected against rickets. Several organic acids were tested but only the citrates and tartrates were found to be effective and these were more beneficial when used as a mixture of the acid and its sodium salt.

Most of these investigations were carried out using the white rat as an experimental animal. Endeavors to apply this information to human subjects have been necessarily limited. Publications by Hanke ('33) and Chaney and Blunt ('25) have shown that in the human being there is a definite retention of calcium and phosphorus after the inclusion of large amounts of orange juice in the diet. Lanford ('39) suggested that the beneficial effects of orange juice, which she too noted, might be due to the citric acid and citrates in such a supplement. Shohl and Butler ('39) tested the value of organic acid therapy on two cases of human rickets. They concluded that mixtures of citric acid and sodium citrate induced healing in infantile rickets without vitamin D supplement.

A search of the literature has failed to reveal the use of any other laboratory subject besides the rat and dog in studying this interesting problem. Within the last few years the chick has become a popular biologic tool to demonstrate differences between the various forms of vitamin D. Thus, Massengale and Nussmeier ('30) and others have demonstrated that chick bone ash is more sensitive to vitamin D in cod liver oil than to that in irradiated ergosterol when used in equal units as standardized on the rat. Likewise Correll and Wise ('38 a, '38 b) found the growth of the chick and the phosphatase concentration in the serum to be

affected by the type of antirachitic agent incorporated into the ration.

It seemed of interest to try and find out if these organic acid-salt mixtures when fed to the chick would produce normal bone ossification even in the absence of vitamin D, or if ammonium chloride-ammonium carbonate would produce rickets with a non-rachitogenic diet as these supplements had been shown to do in the rat. If such results were obtained, what would be the effect on the phosphatase concentration which, supposedly, is directly affected by vitamin D intake? The chick lends itself admirably to further investigation of this problem for, as Massengale and Bills ('36) have pointed out, the production or non-production of rickets in the chick is not dependent upon a favorable mineral ratio. Variations in the calcium-phosphorus content of the diet do not result in an appreciable difference in the bone ash values obtained under a given set of conditions.

It is the purpose of this paper to present the results of our research on these questions.

EXPERIMENTAL

Single comb white Leghorn chicks were started on assay when 1 day old (weight 30 to 35 gm.) and the experiments terminated on the twenty-first day. The birds were divided into groups of fifteen or twenty without regard to sex, each group being kept in a wire-bottomed, electrically-heated battery brooder with constant access to distilled water and the ration.

The basal diet employed was that of Hart, Kline and Keenan ('31), modified to contain fifty-seven parts of maize and two parts of dried yeast. Numerous analyses of this ration in our laboratory have shown it to contain about 1% calcium and 0.7% phosphorus; $\frac{Ca}{P}$ ratio of $\frac{1.4}{1}$. Supplements were made at the expense of the mixed ration as a whole and incorporated so that 100 gm. of feed contained the desired quantity of material to be tested.

Ash determinations and the technique for estimating phosphatase values were carried out according to the methods referred to in previous work (Correll and Wise, '38 a, '38 b).

Every experimental series included negative and positive control groups. This allows for comparison of the results yielded by any group in a series with data from chicks which were rachitic and those that were normal under the conditions of each experiment. It has been noted before (Correll and Wise, '38 a) that bone ash values vary from time to time even under the most uniform conditions. Such fluctuations are of no importance in the interpretation of these tables because positive and negative controls were utilized as indicated.

Since an assay group represents either fifteen or twenty birds, results are recorded as an average of the whole for conservation of space.

RESULTS

The first experiment was designed simply to observe the effects on the bone ash of chicks of certain citrate mixtures when incorporated into a diet known to be rachitogenic for the birds. The salt and acid supplements were identical with those which other investigators had shown to be antirachitic for rats. The quantities fed were planned to approximate the amounts found effective in rats of similar starting weights (30 to 35 gm.).

The results obtained (table 1, experiment I) were unexpected. The only group (citric acid-potassium citrate, group 3) in which a large percentage of the birds lived through the assay period showed poorer growth than the positive controls and complete failure of the supplement to enhance ossification as judged by the bone ash value obtained. The other acid-salt additions, which had been effective for rats, killed the chicks. The citric acid-sodium citrate appeared to be especially toxic (group 5).

This led to an investigation of a series of cationogen and anionogen mixtures. All of these have been reported in the

TABLE 1

The effect of adding cationogens and anionogens to rachitogenic and non-rachitogenic diets for chicks

GROUP NUMBER	SUPPLEMENT PER 100 GM. OF DIET	BIOLOGIC RESPONSE				
		Final weight	Bone ash	Phosphatase	Survival data [1]	"Salt" effects
Experiment I						
		gm.	%	*Units* [2]		
1	Negative controls	89	36.9	*	18	*
2	Positive controls 27 I.U. of D from C.L.O.	107	45.0	*	18	*
3	0.02 mol. citric acid 0.02 mol. potassium citrate	72	36.3	*	18	*
4	0.04 mol. citric acid 0.04 mol. potassium citrate	62	34.0	*	5 Others died 2nd, 3rd wk.	*
5	0.02 mol. citric acid 0.02 mol. sodium citrate	*	*	*	All died 1st wk.	*
Experiment II						
6	Negative controls	90	36.6	165	17	None
7	Positive controls 27 I.U. of D from C.L.O.	136	47.7	52	16	None
8	0.01 mol citric acid 0.025 mol. sodium citrate	*	*	*	All died 1st wk.	+++
9	0.05 mol. tartaric acid 0.03 mol. sodium tartrate	*	*	*	All died 2nd, 3rd wk.	None
10	0.05 mol. maleic acid 0.03 mol. sodium maleate	*	*	*	All died 1st wk.	None
11	0.1 mol. lactic acid 0.06 mol. sodium lactate	*	*	*	All died 1st wk.	+
12	0.1 mol. acetic acid 0.06 mol. sodium acetate	*	*	*	All died 1st wk.	+++
13	0.02 mol. citric acid 0.02 mol. potassium citrate	56	37.0	114	10	None
14	0.02 mol. citric acid 0.02 mol. potassium citrate 27 I.U. of D from C.L.O.	67	41.6	94	11	None
15	0.02 mol. citric acid 0.02 mol. potassium citrate 100 I.U. of D from C.L.O.	98	48.1	35	17	None
16	0.03 mol. ammonium carbonate 0.06 mol. ammonium chloride 27 I.U. of D from C.L.O.	81	45	55	15	None

[1] Twenty chicks started in each group. This column records how many survived, or how rapidly they died.

[2] Bodansky units per 100 cc. of serum.

* Not investigated for this group.

+ Slight "salt" deposits observed in some birds on autopsy.

+++ Heavy "salt" deposits observed in practically all birds on autopsy.

literature as having been fed to rats. Some were effective antirachitic agents, others not. None was reported as toxic to the rat.

The data are tabulated in table 1, experiment II. Citric acid-sodium citrate (group 8) even at a much lower concentration than in the first experiment was extremely toxic to the chick.

Autopsies were performed on these birds and a gross examination made of the internal organs. Heavy, white "salt" deposits were observed in localized areas throughout the body. The femur-tibio-tarsus joint, or knee, was often swollen by a congestion of the precipitate at this juncture. Such findings accounted for the stiff-legged gait noticed in many chicks before death. The pericardium in some cases was so coated as to make the heart appear as a white stone. Irregular patches of white "salt" were seen scattered through the subcutaneous fascia. The kidneys were always affected, appearing as though full of solid material; when cut they were found to contain a milky, gritty, viscous fluid. The ureters were occluded and hard; from them elongated, stone-like pieces were extruded.

The nature of this precipitate has not been established. Stones obtained from the ureters undoubtedly were highly contaminated. Positive qualitative tests were observed for calcium, phosphorus, sodium, potassium, and citrate radical. The material appeared to be practically insoluble in hot or cold water, alcohol, and concentrated HCl, and somewhat soluble in 25% NaOH. One sample yielded 22.8% ash.

The supplements of tartaric, maleic, lactic and acetic acid-salt mixtures (groups 9 to 12 inclusive) also killed the birds, although only with the acetic acid-sodium acetate mixture were appreciable deposits of "salt" noticed.

Again citric acid-potassium citrate (group 13) allowed fair survival and no "salt" was seen in the birds that died. However, this mixture was in no way antirachitic for the chick as demonstrated by the poor growth, low ash, and comparatively high phosphatase results obtained. Indeed such a sup-

plement appeared to interfere with ossification even when cod liver oil was included in the diet. An established adequate amount of vitamin D, under these conditions (group 14), improved the biologic response of the group but still yielded values far below those shown by the positive controls. A large dose of vitamin D (group 15), four to five times the minimum required by chicks, gave only fair growth but the bone ash and phosphatase values were normal.

The anionogens (group 16) added to the basal diet along with vitamin D, failed to produce rickets as measured by the bone ash and phosphatase values. This supplement did not permit good growth; however, it was not rickets-producing for the chick on a non-rachitogenic diet, as a similar supplement has been shown to be for rats on diets which, because of their favorable mineral ratio, were non-rachitogenic for them.

Potassium citrate mixtures were much less toxic than sodium citrate at equivalent levels (compare group 3 with group 5), and even when fed at a greater concentration (group 4) the birds lived longer with no manifestation of "salt" on autopsy. There was also indication that vitamin D "protected" the chicks somewhat from the toxic action which potassium citrate did exert (see group 13, 14 and 15). Such observations gave rise to the question of what influence potassium citrate and vitamin D would have individually and together if included in a ration with the more toxic sodium citrate.

By reference to table 2, experiment III, it will be noted that vitamin D (groups 20 to 23, inclusive) even in massive doses, failed to protect the chick against the toxicity of sodium citrate. Potassium citrate added with the sodium salt in an equal molar quantity (group 24) allowed for normal survival, but this mixture was not antirachitic for the chick as demonstrated by the low bone ash value. Sodium citrate in excess of potassium citrate (group 25) still killed the birds but the "protective" action of the latter salt is noted by the increase in the time of survival and failure to observe

TABLE 2

The effect of feeding vitamin D or potassium citrate with sodium citrate, and of feeding the component parts of sodium and potassium citrates to the chick

GROUP NUMBER	SUPPLEMENT PER 100 GM. OF DIET	BIOLOGIC RESPONSE			
		Final weight	Bone ash	Survival data [1]	"Salt" effects
Experiment III					
		gm.	%		
18	Negative controls	130	37.6	14	None
19	Positive controls 27 I.U. of D from C.L.O.	181	47.7	14	None
20	0.02 mol. sodium citrate	*	*	All died 1st, 2nd wk.	+++
21	0.02 mol. sodium citrate 27 I.U. of D from C.L.O.	*	*	All died 1st wk.	+++
22	0.02 mol. sodium citrate 100 I.U. of D from C.L.O.	*	*	All died 1st, 2nd wk.	+++
23	0.02 mol. sodium citrate 500 I.U. of D from C.L.O.	*	*	All died 1st wk.	+++
24	0.02 mol. sodium citrate 0.02 mol. potassium citrate	101	38.0	13	None
25	0.05 mol. sodium citrate 0.02 mol. potassium citrate	*	*	All died 2nd, 3rd wk.	+
26	0.05 mol. sodium citrate 0.02 mol. potassium citrate 27 I.U. of D from C.L.O.	50	40.0	5 Others died 2nd, 3rd wk.	+
Experiment IV					
27	Negative controls	152	38.2	13	None
28	Positive controls 27 I.U. of D from C.L.O.	155	46.4	15	None
29	0.06 mol. sodium chloride	139	38.7	13	None
30	0.12 mol. sodium chloride	122	37.0	8	None[2]
31	0.06 mol. potassium chloride	112	39.0	15	None
32	0.06 mol. sodium chloride 0.06 mol. potassium chloride	125	40.7	14	None[2]
33	0.04 mol. citric acid	77	33.9	14	None
34	0.02 mol. citric acid 0.06 mol. sodium chloride	120	34.5	15	None
35	0.04 mol. citric acid 0.12 mol. sodium chloride	94	34.2	15	None

[1] Fifteen chicks started in each group. This column records how many survived, or how rapidly they died.

[2] Some edema apparent in these birds.

* Not investigated for this group.

\+ Slight "salt" deposits observed in some birds on autopsy.

+++ Heavy "salt" deposits observed in practically all birds on autopsy.

any significant "salt" deposits in the dead birds. Potassium citrate and vitamin D together (group 26) were yet more "protective" against sodium citrate; if the vitamin D had been included at a higher level the chicks might all have lived.

Experiment IV in table 2 is self-explanatory. Neither sodium nor potassium as chloride, in the lower concentrations, were particularly toxic to the chick; when they were, no "salt" deposits were seen. None of these salt supplements is antirachitic; the slight increase in bone ash noted in group 32 is probably not significant. Citric acid alone (group 33) did not kill the birds, although neither did it allow for good growth. In group 34 sodium chloride and citric acid were added in amounts calculated to produce sodium and the citrate radical equal to that yielded by 0.02 mol. of sodium citrate. Quantities equivalent to 0.04 mol. of sodium citrate were fed to another group (group 35). Under these conditions no toxicity was observed comparable to that demonstrated when 0.02 mol. of sodium citrate was included in the diet. All the chicks lived, but the salts were not antirachitic as shown by the bone ash percentages.

DISCUSSION

It would appear from these results that probably no simple organic acid or its sodium and potassium salt, when added to a rachitogenic diet, will protect the chick from rickets as it does the rat. From one experiment it also seems that ammonium chloride-ammonium carbonate mixture in a ration non-rachitogenic for chicks will not produce rickets in the birds as it has been shown to do with rats on basal diets that were non-rachitogenic because of a favorable mineral ratio.

Most of the cationogens fed to the chicks were toxic, particularly sodium citrate and acetate which caused the precipitation of a "salt" in the animal's body. Potassium citrate seems to "protect" the chick from this toxic manifestation of sodium citrate. Likewise vitamin D allowed the chick greater tolerance for the somewhat toxic potassium citrate. No explanation is offered for these observed facts.

The author is indebted to Mr. Anthony Bucci for his care of the animals and for assistance in the autopsies.

SUMMARY

1. No organic acid, its sodium or potassium salt, or a mixture of the acid and its salt when added to a diet that is rachitogenic for chicks was found to protect the birds from rickets as similar mixtures are known to do for rats.

2. Most of the cationogens studied were toxic to the chick. Sodium citrate and acetate caused the precipitation of an insoluble "salt" in the animal body.

3. When citric acid and sodium chloride were fed in amounts equivalent to a lethal dose of sodium citrate no such toxic manifestations were noted.

4. Potassium citrate is less toxic than sodium citrate. When the two were fed together in an equal molar ratio the chick was "protected" from the greater toxicity of the sodium citrate.

5. Ammonium carbonate-ammonium chloride mixture when added to a non-rachitogenic ration for chicks did not produce rickets, as measured by bone ash and serum phosphatase values, in contrast to results that have been reported for rats on diets which were non-rachitogenic because of a favorable mineral ratio.

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A STUDY OF VITAMIN C NUTRITION IN A GROUP OF SCHOOL CHILDREN

PART II. DIETARY EVALUATION [1]

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ONE FIGURE

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A study of the vitamin C status of a group of eighty-six elementary school children in a Maine village was made during the late fall of 1938 and early spring of 1939, seasons when vitamin C containing foods are less abundant than during the summer months. Most of the children were of French-Canadian extraction, and the dietary habits, food preferences, and cooking methods are characteristic of the native stock of this region only.

The following criteria were used to judge vitamin C nutrition: (1) fall and spring plasma ascorbic acid values as determined by a modification of the Ingall's technique ('37); (2) the results of urinary tolerance tests on forty-nine children, twenty-three of whom were from the group of eighty-six children; (3) oral conditions determined by examination in fall and spring; (4) the effect of vitamin C therapy on gum inflammation among forty-one children, twenty-six of whom were from the group of eighty-six children; (5) analyses of dietary records kept for 1 week by seventy-six children in the autumn and sixty-three children in the spring of whom

[1] Paper 236 of the biological laboratory of the Maine Agricultural Experiment Station.

sixty and forty-six respectively were from the group of eighty-six children;[2] the record of only one child in a family was used giving one record for approximately every five of the 325 families represented at the school.

The children in the study were unselected except for age and sex. The group included thirty-nine boys and forty-seven girls, ranging in age from 7 to 16 years.

The laboratory tests involving the determination of plasma ascorbic acid and urinary tolerance tests were conducted by Dr. Marian M. Crane of the Children's Bureau, Department of Labor, Washington, D. C. The oral examinations were made by Dr. Phillip W. Woods of the Dental Division, Bureau of Health, Augusta, Maine. It is pertinent to the present report to summarize their results briefly as follows:

RESULTS OF THE CLINICAL AND LABORATORY STUDIES[3]

1. Approximately 45% of the children in the autumn and nearly 63% in the spring manifested plasma ascorbic acid values of less than 0.40 mg. per 100 ml. Only two children of the group manifested values of 0.80 mg. per 100 ml. or above at both autumn and spring examinations.

2. The urinary tolerance tests demonstrated that the twenty-eight children whose plasma ascorbic acid values were less than 0.40 mg. per cent all excreted less than 10% of a 400 mg. dose of ascorbic acid within 6 hours after ingestion. In contrast, the four children with plasma values 0.80 mg. per cent or above excreted more than 10%, in three cases 30% or above.

3. Oral examinations showed gum inflammation in slightly more than one-third of the children in the autumn and about one-half of the children in the spring. Of the twenty-four

[2] A number of additional records obtained in the spring could not be included because the families had recently received grapefruit from the Federal Surplus Commodities Corporation. The records were, therefore, not considered to represent the child's usual diet.

[3] Crane, Marian M., and Phillip W. Woods. 1941. A study of vitamin C nutrition in a group of school children. Part I. Clinical and laboratory studies. New England J. Med., vol. 224, p. 503.

children with low plasma ascorbic acid values (below 0.40 mg. per cent) both in autumn and spring, only three were free from gum inflammation at both examinations. Of the seventeen children with consistently higher plasma values (0.40 mg. per cent or above), ten were entirely free from inflammation.

4. Of the forty-one children included in the therapy test, six showed extensive, nineteen moderate, and sixteen slight gum inflammation. The children were instructed to take orally 200 mg. of crystalline ascorbic acid daily, and were re-examined from 19 to 21 days after therapy was started. About two-thirds (twenty-eight) of the forty-one children showed improvement in oral condition, thirteen being free from inflammation. About one-third (thirteen children) showed no change in oral condition.

DIETARY EVALUATION IN TERMS OF VITAMIN C

Food records

Concurrent with the observations reported above on plasma ascorbic acid values and oral conditions of the school children, a study of dietary habits and food values was carried out.

The frequencies with which vitamin C containing foods (except potato which is discussed in a separate section) were reported is shown graphically in figure 1.

Of the foods commonly eaten in the United States, citrus fruit is the best source of vitamin C. In the autumn only seventeen of the seventy-six children ate any citrus fruit during the week for which the record was kept; in the spring half of the children had some citrus fruit but only fourteen of the sixty-three had it oftener than once or twice in the week.

Other foods reported which are good sources of vitamin C [4] are tomatoes, cabbages, and turnips (rutabaga). These foods,

[4] Greens, which are usually considered a good source of vitamin C, have not been included as such here. Chemical analyses of greens, after preparation for consumption according to the local methods, showed their vitamin C content to be relatively low.

as well as citrus fruits, were lacking from the diets of fifteen of seventy-six children in the autumn and ten of sixty-three children in the spring, and they were eaten only once or twice a week by twenty-nine children in the autumn and fifteen in the spring. At neither season did more than one child in seven have one good source of vitamin C each day, the minimal recommendation for a satisfactory diet (Children's Bureau Folder, '39).

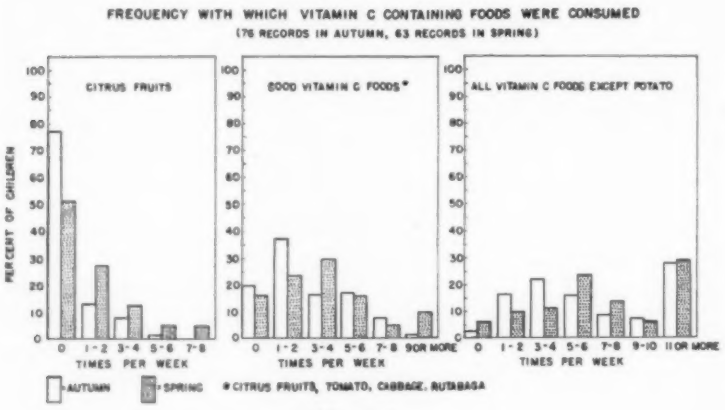
FREQUENCY WITH WHICH VITAMIN C CONTAINING FOODS WERE CONSUMED
(76 RECORDS IN AUTUMN, 63 RECORDS IN SPRING)
CITRUS FRUITS
GOOD VITAMIN C FOODS*
ALL VITAMIN C FOODS EXCEPT POTATO
PER CENT OF CHILDREN
0 1-2 3-4 5-6 7-8 9 OR MORE 9-10 11 OR MORE
TIMES PER WEEK
AUTUMN
SPRING
* CITRUS FRUITS, TOMATO, CABBAGE, RUTABAGA

Figure 1

The other foods supplying vitamin C that were included in the diets studied are only fair sources of this factor. There were at least half of the children (forty-three of seventy-six in the autumn, thirty-two of sixty-three in the spring) who did not eat any vitamin C containing food other than potato as often as once a day and about one-sixth who did not eat such food more than twice during the week.

Figure 1 shows that many of the diets were markedly inadequate in respect to vitamin C containing foods. Only 14% in the spring and 9% in the fall included one food which is a good source of vitamin C daily, while 40% in the spring and 58% in the fall reported such a food only twice a week or even less.

Food analyses

While conducting this vitamin C survey in the State of Maine, it was found necessary to perform a certain number of analyses for vitamin C in order to evaluate the diets of these subjects. In the first place, many of the foods reported were substances whose vitamin C contents had never been determined and which seemed unique to the area. Secondly, cooking methods in that particular district differed from usual techniques and the data for cooked foods found in the literature were not applicable to the foods consumed in the district. Thirdly, investigations at the biological laboratory of the Maine Agricultural Experiment Station had shown that locally-grown produce varied considerably as to vitamin C values from that produced elsewhere (Murphy, '39).

METHOD

To investigate destruction of vitamin C by home cooking, a frequency distribution was made of the cooking technique data obtained on visits to each home. These data included length of cooking time, amount of water used, type of cooking utensil, and method of preparation of vegetables before cooking.

On the basis of this information, several of the homes where typical methods were used were visited. Procedures were observed with a view of duplication in a fully equipped laboratory. Samples of the cooked foods [5] were taken to the temporary field laboratory and analyzed for reduced ascorbic acid using the Bessey and King ('33) titrimetric method.

When duplicate analyses were performed at the permanent laboratory at Orono, it was found that all of the foods under observation contained some of the available vitamin C in the dehydro form. For that reason and for purposes of brevity, most of the data obtained in the field laboratory on reduced ascorbic acid are not included in this paper.

[5] Chemical analysis of the cooking water was omitted, since, in the homes, it was always discarded.

EXPERIMENTAL RESULTS

Potatoes. The frequency with which potatoes were eaten by the individuals under observation and the rarity of other vitamin C containing foods in the diets place this vegetable in the position of a primary source of vitamin C for this particular region. Following is an analysis of the diet records.

NUMBER OF TIMES PER WEEK POTATOES WERE EATEN	PERCENTAGE OF DIET RECORDS LISTING POTATOES WITH SPECIFIED FREQUENCY	
	Autumn (76 records)	Spring (63 records)
	%	%
0– 4	9.2	15.9
5– 9	68.4	71.4
10–13	22.4	12.7

Thus the losses of ascorbic acid sustained during cooking and storage, while small in themselves, constitute an important loss to the consumer, who is relatively dependent upon this supply.

Locally grown Green Mountain potatoes stored under the usual storage conditions of the region and cooked according to the local custom were analyzed for total ascorbic acid using the technique published by Reedman and McHenry ('38). Reductions with H_2S were carried out on all samples, raw and cooked, as this was found to be necessary—the per cent of dehydro ascorbic acid varying from 16% to 68% of the reduced. The data are tabulated in table 1.

Cabbage. Cabbage is an excellent source of vitamin C. It may be grown readily in all parts of Maine and may be stored for winter consumption. Of even more importance, it is a familiar food which should require no educative encouragement for its inclusion in the dietary. It was chosen by only nineteen of ninety-one children in the spring and by eighteen of seventy-six children in the fall. Of these, four of the fall group and five of the spring group consumed it raw.

Varietal differences in Maine grown cabbages were found to be of interest. As nearly as could be determined by inquiry, the Ballhead varieties are the most commonly grown in the region. In analyzing for varietal differences, fourteen varieties

TABLE 1

Ascorbic acid content of fresh foods reported on dietaries. All foods were cooked in covered utensils and in large amounts of water except as otherwise noted

MATERIAL AND SAMPLING TECHNIQUE	METHODS OF PREPARATION	TOTAL ASCORBIC ACID MG. PER GM.		PER CENT RETENTION OF ORIGINAL RAW VALUE
		Raw	Cooked	
Potato. Green Mountains	Raw	0.17		
Fresh, locally grown. Fall	Unpeeled. Boiled 30 min.		0.10	59
Radial sectors from three potatoes	Peeled. Boiled 30 min.		0.10	59
Stored 8 months in local potato house. Spring	Raw	0.11		
	Unpeeled. Boiled 30 min.		0.06	55
	Peeled. Boiled 30 min.		0.05	45
	Baked 400°F. 39 min.		0.07	64
	Fried raw 15 min.		0.08	73
Cabbage. Bugner Ballhead variety. Locally grown	Raw	0.70		
Raw samples from center cross sections of three cabbages				
Cooked samples from quartered sections of three cabbages	Boiled 30 min.		0.38	54
	Boiled 1 hour		0.31	44
	Boiled 2 hours		0.23	33
From market. Variety unknown	Raw	0.32 [1]		
	Roasted 1 hour and 55 min. with beef			
	Leaves separated		0.11 [1]	34
Rutabaga. From market				
Variety unknown. Radial sectors of cross sections of 3 rutabagas	Raw	0.44 [1]		
Approx. 1 in. strips, ¾ in. thick	Boiled 25 min. Small amount of water		0.38 [1]	86
Approx. 2 in. squares, ½ in. thick.	Boiled 1½ hours		0.22 [1]	50
Fiddlehead greens (Onoclea struthiopteris)	Raw	0.45		
Native wild greens, gathered locally	Boiled 30 min. Small amount of water		0.32	71
Sample—14 heads	Boiled 2 hours		0.20	44

[1] Dehydro ascorbic acid not determined. Figures represent reduced values.

of cabbages were grown at Orono, Maine during the summer of 1939.

The values for mature cabbages consist of an average of from three to five samples of each variety covering a period of from 6 to 10 weeks. At maturity, the average amounts of reduced ascorbic acid in cabbage types were as follows:

TYPE	VARIETIES	MG. PER GM.
Ballhead	3	0.48
Early Savoy	3	0.46
Miscellaneous, Late	2	0.43
Early Wakefield	2	0.43
Midseason	2	0.42
Early Copenhagen	2	0.36

Losses sustained during cooking were investigated. Of ninety-three housewives interviewed, only seventy reported cooking techniques for cabbages. The lengths of the cooking periods reported most frequently were 30, 60 and 120 minutes. Complete analytic data are recorded in table 1.

Rutabaga. Rutabaga was found to be a relatively potent source of ascorbic acid. To it may be ascribed all of the advantages enumerated for cabbage, viz., facility of production and storage and familiarity to the consumer. This vegetable was reported by fourteen of ninety-one children in the spring and by eighteen of seventy-six children in the autumn. The amounts of ascorbic acid existing in nine varieties of Maine-grown rutabaga ranged from 0.44 mg. per gram for the Macomber variety to 0.61 mg. per gram for the Large White French variety (McIntosh, '38), thus making the choice or variety to be produced and/or consumed an important one.

Data resulting from the analyses of rutabaga are recorded in table 1.

Greens. Greens were reported by twelve of ninety-one children in the spring and eleven of seventy-six children in the fall. Most of the greens reported consisted of the home-canned or home-salted types which furnish very little ascorbic acid as shown in table 2. Fiddlehead greens, a wild

native green which is a very popular dietary item during May and June, were chosen as typical greens to demonstrate losses incurred during cooking according to the methods in use. Cooking losses are shown in table 1.

Tomatoes. In the spring the ninety-one children reported eating tomato products (raw, canned, or juice) seventy-eight times and in the fall seventy-six children reported them fifty-five times. These products were obtained on the market. The most popular brands were analyzed and the data are recorded in table 2.

TABLE 2

Ascorbic acid content of canned foods reported on dietaries

MATERIAL	METHOD OF PREPARATION	TOTAL ASCORBIC ACID MG. PER GM. AS TAKEN FROM JARS
Home-salted greens: spinach, beet, and turnip tops	Raw greens and dry salt	0
Home-canned fiddle-head greens	No record. Obviously cooked.	0.04 [1]
Home-canned turnip greens	Cooked 2½ hours	0.07 [2]
Home-canned cultivated strawberries	1 part sugar to 1 part berries. Simmer 6 min. Set in sun for 4 days. Pack	0.06
Home-canned field strawberries	1 part sugar to 1 part berries. Stand overnight. Warm on back of stove. Pack	0.04
Home-canned field strawberries	1 part sugar to 1 part berries. Stand 24 hours. Seal in hot jars	0.04
Home-canned raw rhubarb	Rhubarb and cold water	0.06
Home-canned rhubarb sauce	Raw and sugar. Heated 10–15 min.	0.06
Tomato juice, market	Brand A	0.15
Canned tomatoes, market	Brand B	0.12
Canned tomatoes, market	Brand C	0.11

[1] Fifty per cent loss when heated to serve.

[2] Forty-three per cent loss when heated to serve.

Undoubtedly during the growing season fresh tomatoes furnish a more substantial part of the vitamin C supply as they are produced to a small extent in the region. The brevity of the growing season necessitates an early variety. A typical variety grown in the locality is Penn State Earliana. This

variety was produced and analyzed at Orono, Maine during the summers of 1938 and 1939. Of fifteen samples tested, the values ranged from 0.17 to 0.25 with a mean of 0.21 mg. per gram for ripe fresh tomatoes. This was the second lowest value obtained of twenty-four varieties tested. The mean values for all of the tomato varieties analyzed ranged from 0.17 mg. per gram to 0.33 mg. per gram. An early high vitamin C variety of tomato adapted to the soil and climate would be a desirable substitute for the Earliana.

Apples. In the spring, thirty-eight of ninety-one children and forty of seventy-six in the autumn reported the consumption of raw apples. It was not possible to determine the variety which was most frequently eaten. Determinations have shown that certain varieties of apples contain five times as much vitamin C as do others (Murphy, '39). In view of these large varietal differences the exchange of one variety for another would deprive or assure Maine people of part of their yearly vitamin C supply (Dove and Murphy, '36).

Miscellaneous. Other vitamin C containing foods occurring in the dietaries were found not to contain very large amounts of this food essential. Canned field strawberries and canned rhubarb were analyzed and the resulting data are tabulated in table 2.

DISCUSSION

On the whole, analyses of the food records and determinations of the vitamin C values of the foods involved, demonstrate the inadequacy of this factor in the dietaries.

The above figures tend to show that potatoes, especially in the fall, may provide a valuable part of the daily vitamin C requirement. Smith ('38) has tentatively suggested that 50 mg. daily would supply the usual needs of an adult with but little margin of safety. If potatoes are the sole source of vitamin C, to provide a daily intake of 50 mg. would necessitate eating seven average-sized (5½ ounces) peeled, boiled potatoes per day in the spring. This would represent a calorie intake of slightly less than 900 calories. In the autumn three and one-

half boiled potatoes daily would provide a similar amount of ascorbic acid, representing somewhat less than 500 calories.

Over two-thirds of the children were eating potatoes about once a day and were obtaining from this source about 15 mg. of ascorbic acid daily in the fall and 8 mg. daily in the spring. These amounts, although not considered adequate for optimum health, were perhaps large enough to prevent the manifestation of the usually recognized symptoms of clinical scurvy. On the other hand, the low plasma values, as well as the prevalence of gum inflammation, indicate that potatoes were not supplying enough vitamin C to make the inclusion of other vegetables and fruits in the diet unimportant.

In native-grown and home-stored cabbage lies a potential supply of vitamin C in amounts large enough to furnish adequately man's requirements without over-emphasizing the place of cabbage in the diet. More frequent consumption of cabbage in the raw state would enhance the nutritional value received. Losses incurred during the cooking process are large but even after 2 hours of boiling, 23 mg. per 100 gm. of vitamin C remain. By using shorter cooking times and diminishing the amount of cooking water, the vitamin C remaining available in cabbages ranged from 31 to 38 mg. per 100 gm. Vitamin C needs could be supplied with an intake of less than 100 calories from cooked cabbages.

Rutabaga also is a locally produced and stored vegetable which could provide a very substantial portion of vitamin C needs.

Fresh greens in the raw state are reported by Burrell and Miller ('39), Fenton, Tressler, Camp and King ('37), and Tressler, Mack and King ('36) to contain relatively large amounts of ascorbic acid. Greens cooked for long periods of time and in large amounts of water cannot be depended upon to contribute materially to vitamin C requirements. The home-canned greens were found to be of little value and salted greens of no value in furnishing this food essential during the winter period of shortage.

Raw and canned tomatoes could easily be utilized to good advantage in providing vitamin C. In this respect it may be well to point out that certain varieties of tomatoes furnish twice as much of the vitamin for a given weight of tomato as do other varieties. If the original content is high, obviously the amount remaining after processing would be of more value to the consumer. Therefore, nutritional superiority, as well as adaptability to soil and climate, should be considered an important criterion in evaluating varieties of fruits and vegetables to be produced for home or market consumption.

The same qualifications may be applied when considering apples as a source of vitamin C. The choice of variety is all-important if the consumer depends on apples for part of his daily supply.

SUMMARY

A study of vitamin C nutrition, as judged by plasma ascorbic acid values and by oral conditions reported elsewhere has been amplified in this report by a study of the diet records and by determinations of total ascorbic acid in the foods consumed by the children under observation.

Analyses of seventy-six fall and sixty-three spring diet records showed that only one child in seven attained the minimum dietary standard of one good vitamin C food daily.

Storage losses were demonstrated in potatoes, the chief source of vitamin C in the dietaries observed. The ascorbic acid values of raw Green Mountain potatoes decreased from 0.17 mg. per gram in the fall to 0.11 mg. per gram in the spring.

Losses during cooking, according to the techniques employed in the region, were as follows: for potatoes, 27 to 55%; for cabbages, 46 to 67%; for rutabagas, 14 to 50%; and for fiddlehead greens, 29 to 56%.

The findings of vitamin C undernutrition in which 45 and 63% of the children examined showed plasma ascorbic acid values of less than 0.40 mg. per cent, while 32 and 51% of

the children showed oral inflammation, are here shown to be paralleled by markedly inadequate vitamin C intakes in 58 and 40% of the dietaries examined. The large losses of vitamin C attributed to the effects of storage and poor cooking methods emphasize the dietary relation to the clinical symptoms.

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